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Laboratory Feeding and Growth of Juvenile Sablefish, *Anoplopoma fimbria*

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Shenker, J. M., and B. L. Olla. 1986. Laboratory feeding and growth of juvenile sablefish, *Anoplopoma fimbria*. Can. J. Fish. Aquat. Sci. 43: 930–937.

We collected wild juvenile sablefish (*Anoplopoma fimbria*) from the neuston off Oregon and established them in laboratory culture. Growth was determined weekly for up to 8 wk for fish over a size range of 35–110 mm SL (0.3–16.5 g) when provided with one of two live prey species at four ration levels. Fish were fed either mysid shrimp, *Archaeomysis grebnitzkii*, or adult brine shrimp, *Artemia salina*, at ration sizes of 10, 20, and 30% wet body weight/d, and with ad libitum availability of food. Fish provided with mysids at a given ration size generally had mean daily length and weight increments higher than those achieved with *Artemia* at the same ration. Superiority of the mysids for promoting growth was primarily due to their higher dry to wet weight ratio. Gross conversion efficiencies exceeding 40% were achieved on low rations of mysid prey. Peak growth exceeding 2 mm/d and 1.0 g/d establishes juvenile neustonic sablefish among the fastest growing juvenile fishes yet examined.

Nous avons prélevé dans le neuston de jeunes morues charbonnières (*Anoplopoma fimbria*) au large de l'Oregon et nous les avons élevées en laboratoire. On a déterminé à chaque semaine et cela pendant une période allant jusqu'à huit semaines la croissance des poissons ayant une LS variant de 35 à 110 mm (0,3 à 16,5 g) pendant qu'on leur donnait l'une des deux espèces de proie vivantes suivant quatre niveaux de rationnement. On a nourri les poissons soit de mysis (*Archaeomysis grebnitzkii*) ou d'artimias adultes (*Artemia salina*) à des rations correspondant à 10, 20 et 30 % du poids humide corporel/jour, et en leur donnant de la nourriture à volonté. De façon générale, on a noté chez les poissons qu'on a nourri de mysis à une ration donnée une augmentation moyenne quotidienne de longueur et de poids plus élevée que celles obtenues avec *Artemia* pour une même quantité de nourriture. Les mysis ont favorisé davantage la croissance en raison surtout du fait que le rapport poids sec / poids humide de leur corps est plus élevé. On a obtenu une efficacité de transformation de nourriture de plus de 40 % lorsqu'on donnait des mysis en petites quantités. Une croissance maximale dépassant 2 mm et 1,0 g/jour classe les jeunes morues charbonnières faisant partie du neuston parmi les jeunes poissons examinés jusqu'ici qui croissent le plus rapidement.

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The sablefish, *Anoplopoma fimbria*, is a commercially important species occurring along the continental shelves and slopes from Mexico to the Bering Sea to northern Japan (Hart 1973). Spawning of large (1.8–2.2 mm) eggs reportedly occurs from January through April, with peak activity off British Columbia occurring in February at depths below 300 m (Mason et al. 1983). Incubation time is unknown, but larvae are reported to appear in the plankton in March and April, and are found from coastal waters to more than 300 km offshore (Richardson et al. 1980; Mason et al. 1983; Kendall and Clark 1982). Most larvae and juveniles were collected with neuston nets, and have rarely been taken in subsurface plankton collections. Larger juveniles up to 250 mm are also found near the surface, often in association with floating objects, and are attracted to ship-board night-lighting systems (Mitchell and Hunter 1967; Grinols and Gill 1968; pers. obs.). The juveniles eventually settle to the bottom, with adults most common at depths of 350–1000 m (Bourne and Pope 1969). Although adults have a maximum life span of about 40 yr, they approach an asymptotic length of 50–70 cm within their first 10–15 yr (Beamish and Chilton 1982).

Our initial objectives were to determine the feasibility of collecting early juvenile sablefish and establishing them in laboratory culture. We subsequently characterized growth patterns of juveniles fed at four ration levels on two types of live prey.

Materials and Methods

Collection and Experimental Procedures

Sablefish for the preliminary study were collected from the neuston at night during a 4-d cruise aboard the Soviet R/V *Equator* in early May 1983 off the north and central Oregon coast. In 1984, juvenile sablefish were routinely collected during a survey of the neustonic ichthyoplankton and meroplankton off the central Oregon coast (J. M. Shenker, Bodega Marine Laboratory, Bodega Bay, CA 94923, unpubl. data). Healthy juveniles were removed from the cod end buckets of the neuston nets and immediately placed in water-filled plastic

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TABLE 1. Size and composition of prey species used in sablefish feeding experiments.

	Artemia salina	*Archaeomysis grebnitzkii*
Range in mean weight (g)	0.0024–0.0044	0.0115–0.0175
Dry weight (% wet weight); $\bar{X}$ (SD)	11.3 (0.22)	25.5 (2.40)
Lipid (% wet weight)	0.9	2.5
Carbon (% dry weight); $\bar{X}$ (SD)	45.83 (2.28)	43.58 (1.00)
Nitrogen (% dry weight); $\bar{X}$ (SD)	10.68 (0.25)	11.41 (0.17)

bags. The bags were then sealed and floated in ambient temperature (10–14°C) seawater in a fiberglass cooler. Water in the bags was exchanged approximately every 6 h until the fish were returned to the laboratory.

In the laboratory in both years, specimens were maintained in individual aquaria at 13.0 ± 0.5°C, in water of 28–30‰ salinity. A 14 h light : 10 h dark photoperiod, approximating the springtime daylength, was provided with illumination intensities of 1950 and <10 lx, respectively. The aquaria were shielded from disturbance by observers by black plastic sheeting with small holes for viewing. Food was introduced through PVC tubes extending from outside the sheeting to holes in the tank covers.

To monitor the growth of fish in 1983, we placed fish in 10-L aquaria and provided adult *Artemia salina* ad libitum for 20 d. At approximately 7-d intervals, each fish was briefly placed in a narrow glass aquarium (15 × 9 × 4 cm) with a measuring grid on a movable back partition. The grid was moved to within 1 cm of the front of the tank and the total length of the fish was measured. Standard lengths of these fish were calculated using a standard length – total length regression developed from fish examined the following year. All length data is presented as standard length.

In 1984 the growth of juvenile sablefish was studied for a variety of ration sizes using two types of live prey species for food. Specimens were collected on three cruises in April, May, and June and were kept in 40-L tanks equipped with external filters. Each fish was provided with approximately 0.1 g/d of live *Artemia* (40–50 animals) for an acclimation period of 1–7 d.

At the start of the experiment, and approximately every 7 d throughout the study, individuals were anesthetized in a solution of 0.5 g MS-222/4 L of seawater, placed on blotter paper on a Mettler PC 4400 balance, and weighed to the nearest 0.01 g. Standard and total lengths were determined to the nearest 0.1 mm using a dial caliper. The fish were placed in a beaker of fresh seawater for recovery and then transferred back to their individual aquaria. Lights in the laboratory room were extinguished during the measuring procedure to help minimize shock and handling stress. A flashlight was used to provide light while measuring fish. A 30- to 45-s exposure was sufficient to immobilize the specimens. Recovery usually required 1–3 min, with fish readily consuming food 1–2 h after anesthetization.

The first fish brought to the laboratory were provided diets of adult *Artemia*, with ration sizes of 20 and 30% body weight/d. Subsequent fish added to the experiment were given 10% bw/d or ad libitum diets. Other fish were established on similar-sized rations using the mysid shrimp *Archaeomysis grebnitzkii* as prey. Several fish were initially started on *Artemia* and then switched to mysids to monitor the effect of the change in diet on growth. For convenience, these dietary groupings are signified by initial (A = brine shrimp, *Artemia salina*, M = mysid shrimp, *Archaeomysis grebnitzkii*) and ration level (10, 20, 30, and Ad). For example, A20 refers to a diet of *Artemia* at 20% bw/d, and MAd is an ad libitum diet of mysids.

Fish were fed two or three times during each daytime period, depending on the size of the meal. Consumption of each meal was generally completed in 10–15 min. On the day before size measurements were taken, the final meal was presented 14–16 h before the anesthetization procedure was to begin. This allowed a constant time for digestion of food to occur before weighing. Fish on an ad libitum diet were not deprived of food, however, and their weight data thus includes the weight of freshly ingested food.

Because the fish were growing rapidly, it was necessary to provide a daily increase in the ration of each fish to maintain the fish on a constant weight-specific ration. Fish receiving a 30% bw/d ration had their ration boosted by 10%/d. Fish on 20 and 10% bw/d rations had their daily food increased by 5%/d and 5%/2 d, respectively.

To avoid the influence of small tank size on growth (Theilacker 1980), experiments were usually terminated when fish reached 95–110 mm SL. Several fish fed on mysid diets were removed from the experiment before reaching the target size because of increasing difficulty in collecting sufficient mysids as the season progressed.

Shipments of adult *Artemia* were obtained weekly from a commercial supplier. Weights of 100 shrimp from each batch were determined by blotting dry 10 groups of 10 individuals and then weighing the groups to the nearest 0.001 g on a Mettler AC 100 balance. Ration sizes for the fish were then computed using the mean wet weight per prey in a batch to determine the number of prey to be fed to the fish. Live mysids were collected 1–3 times/wk with a fine-mesh dip net from sandy-bottom tide pools on the exposed ocean beach. Fifty individual mysids were blotted dry and weighed from each collection, and the mean weight was again used to determine the number of prey comprising the daily ration.

Four batches of *Artemia* and mysids were dried at 65°C for 48 h to determine the dry to wet weight relationships of each prey type. Three dried samples of each prey species were then analyzed on a Perkin-Elmer model 240B C-H-N analyzer to determine carbon and nitrogen content. In addition, a batch of 50 *Artemia* and a batch of 3 mysids were frozen at 20°C and then later thawed, homogenized, and assayed to determine lipid content of the prey items (Folch et al. 1957; Marsh and Weinstein 1966). Mysids had higher wet weights and percent dry weights and lipid content than did *Artemia* (Table 1).

Analytical Procedures

Growth was expressed as daily increments in SL and weight for each weekly period between measurements. Data from fish on identical diets were pooled for analysis. The effect of size on growth was examined by regression analysis, with the arithmetic mean SL or weight of each fish during the weekly intervals between measurements used as independent variables. Regressions were plotted only over the size range of the fish within the feeding group. Tests of similarity of the regression lines among dietary groups were conducted by calculation of the *t*-statistic from the ANOVA regression components and

TABLE 2. Summary of experiments and size relationships of juvenile sablefish. Diet types are defined in text. All length–weight regressions have $r^2 \geq 0.98$. D = duration of trials (days); p, q = regression parameters: wt = $(p \times 10^{-6})\,SL^q$.

		Range of specimen sizes						
		Start		Finish			Length–weight regressions	
Diet	N	SL (mm)	wt (g)	SL (mm)	wt (g)	D	p	q
A10	4	40.3–61.8	0.55–2.24	52.3–82.3	1.33–5.39	41	6.72	3.07
A20[a]	6	40.6–48.7	0.51–0.91	61.5–88.3	1.97–7.54	22–66	1.35	3.46
A30[b]	7	35.5–52.5	0.26–1.13	67.8–91.8	2.67–9.27	22–46	0.96	3.56
AAd	3	42.5–67.0	0.67–2.54	95.5–110.2	9.50–14.53	21–28	2.83	3.29
M10	4	50.2–62.4	1.16–2.04	68.2–80.5	2.85–4.49	21–30	15.55	2.87
M20[a]	5	46.9–82.4	0.95–5.98	79.1–97.7	5.12–11.90	21	1.94	3.39
M30[b]	3	51.6–80.4	1.11–5.59	94.0–109.8	8.78–16.55	16–21	0.89	3.56
MAd	2	48.3–51.9	0.91–1.23	93.7–100.5	8.99–11.04	21	1.85	3.40
AAd[c]	4	21–33	—	58–76	—	20	—	—

[a]Two fish switched from A20 to M20 after 21 d.
[b]Two fish switched from A30 to M30 after 21 d.
[c]Preliminary trials in 1983; only length data were collected.

slope and intercept coefficients (Neter and Wasserman 1974).

The weight of food given each fish each week was determined by summing the numbers of prey consumed, and multiplying by the mean prey weight. This weight was then compared with the targeted ration. For *Artemia*, the mean measured ration sizes were 10.1 (SD = 0.1), 19.0 (SD = 0.5), and 29.7% (SD = 1.1) bw/d. Mysid ration sizes were 9.5 (SD = 0.1), 18.2 (SD = 0.6), and 28.2% (SD = 0.7) bw/d.

Gross conversion efficiency (Brett and Groves 1979) was computed over the experiment as

$$k_1 = (TF/WG) \cdot 100\%$$

where k_1 = gross conversion efficiency, TF = total weight of food provided during the experiment, and WG = total weight gain of the fish.

Results

Growth

In 1983, four early juvenile sablefish grew from initial lengths of 21–33 mm to 58–76 mm following 20 d of ad libitum feeding on adult *Artemia*. Thirty juveniles tested in 1984 had initial lengths of 35–67 mm and weights of 0.26–2.54 g. They were maintained on the three measured diets or on ad libitum rations for periods of 21–66 d, until sizes of 52–110 mm (1.33–16.55 g) were reached (Table 2).

Fish on all diets other than the minimal A10 ration grew throughout the experiment. The fish fed on A10 grew slowly at the start of the experiment, but growth tapered off as the feeding progressed. Daily increments in length of all four fish decreased over the 41-d observation period. Conversely, fish fed on M10 maintained a relatively constant daily length increment throughout the experiment (Fig. 1a). Daily weight gains of the three small fish on A10 decreased, although the one larger specimen of the group had an increasing daily weight gain. All fish on M10 had daily weight gains that increased as the fish grew (Fig. 1b).

Fish grew faster in length when fed larger rations, with mysid-fed fish growing faster than fish fed the equivalent measured rations of *Artemia* (Table 3). Growth achieved with ad libitum feeding was similar for both prey types. Daily growth of fish fed on a given ration of mysid prey was similar to the growth achieved at the next higher ration of *Artemia* prey (Wilcoxon rank sum comparison, Snedecor and Cochran 1980). Growth on diets of M10–A20, M20–A30, and M30–MAd–AAd were not significantly different ($P < 0.05$) from each other. Regression of daily increment in length (DI) against dry weight of ration size (RS) as percent of wet weight of fish indicated a strong association between the variables, regardless of prey species (DI = 0.181 + 0.879·ln RS, $r^2 = 0.995$; Fig. 2).

The overall mean growth of fish raised on an ad libitum diet of *Artemia* in 1983 was similar to that observed in 1984, but daily rates were more variable (Table 3). The small fish initially averaged 3.0 mm/d, with mean growth decreasing to 1.7 mm/d after 14–20 d.

Daily growth in weight for each feeding group other than A10 increased as the fish grew. These relationships were best summarized by power regression models (Fig. 3). Greater growth occurred at higher ration levels for a given prey type, and for mysid-fed fish at a given ration level than *Artemia*-fed specimens. Analysis of regressions on log-transformed data indicated that daily growth in weight was similar between fish on diets of M10–A20 and M30–MAd–AAd ($P < 0.05$).

The faster growth achieved with mysid prey over *Artemia* prey was further illustrated with fish that were switched from *Artemia* to mysid diets. Two fish on a 20% bw/d diet increased their mean daily length increments from 0.91 (SD = 0.23) to 1.36 mm/d (SD = 0.32) following the prey shift. At 30% bw/d, the mean daily length increment of two fish rose from 1.23 (SD = 0.29) to 1.91 mm/d (SD = 0.14). Daily weight gain also increased sharply following the dietary change.

The power coefficients of the length–weight regressions for each group of fish (Table 2) indicated that allometric growth increasing body robustness (indicated by a regression exponent >3) was achieved on most diets. Fish provided with diets ≥20% bw/d had regression exponents ranging from 3.29 to 3.56, although distinct patterns among the diets were not apparent. For the lowest rations (A10 and M10), the regression exponents were 3.07 and 2.87, respectively, and were indicative of isometric growth.

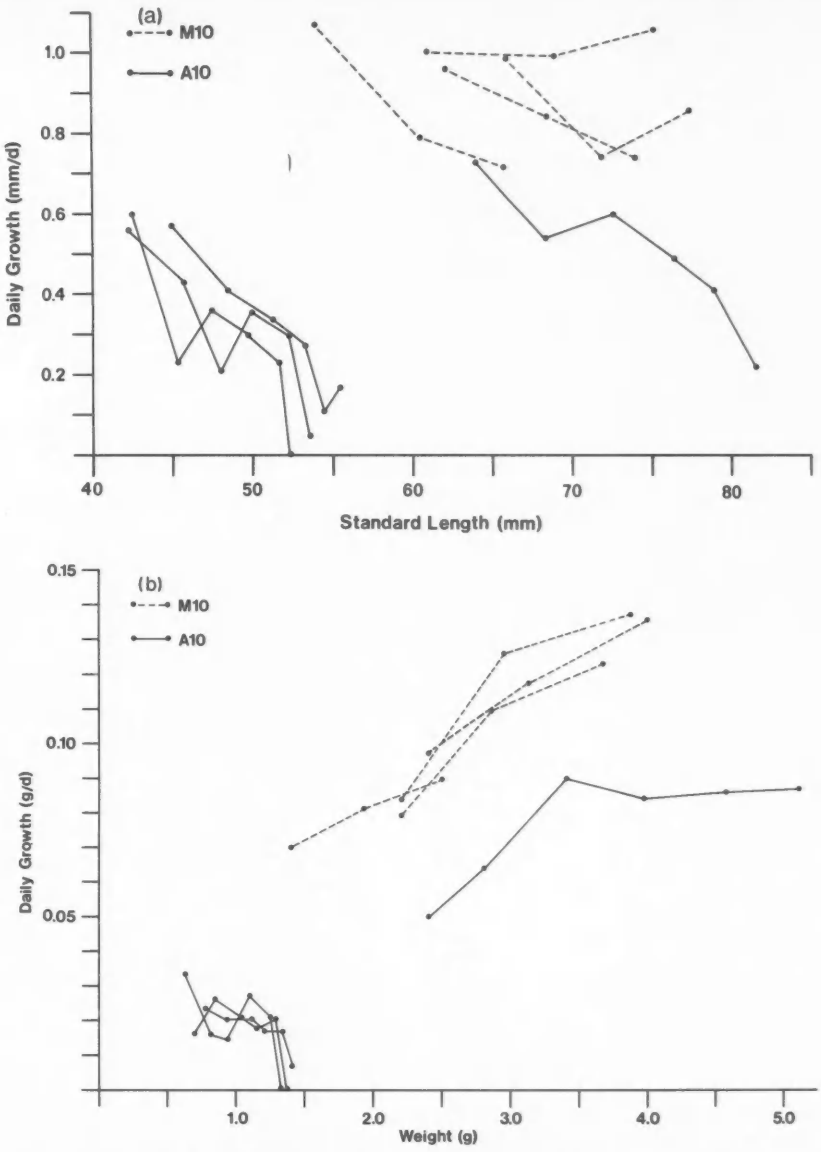

FIG. 1. (a) Daily growth in length of individual sablefish fed on mysids (M10) or *Artemia* (A10) at a ration of 10% bw/d. Each data point indicates mean daily growth over an approximate 7-d period. (b) Daily growth in weight of individual fish fed on M10 and A10 diets.

Gross Conversion Efficiency

The conversion of food into somatic tissue was much more efficient in fish fed on mysid prey than with *Artemia* prey (Fig. 4). At the lowest ration level of mysid prey, the mean conversion efficiency exceeded 40%, with decreasing mean efficiencies at higher ration levels. However, conversion efficiencies between M10–M20 and M20–M30 were not significantly different (Wilcoxon sign rank test, $P < 0.05$). The mean conversion efficiencies achieved on *Artemia* prey ranged from 18 to 23%, but A10–A30 and A20–A30 comparisons were not significantly different ($P < 0.05$).

Discussion

Juvenile sablefish were capable of consuming large quantities of food per day and efficiently assimilating it into somatic tissue. The superiority of mysids over *Artemia* for promoting growth was evident at all measured ration levels, and was particularly striking in fish fed on the smallest ration. The differences in growth between mysid-fed and *Artemia*-fed fish appear to be primarily due to the differences in the water content of the prey items (Table 1), although other nutritional components of the prey, such as lipid content, chitin content, and other unmeasured parameters, may have had an effect on growth.

TABLE 3. Mean (SD) daily length increments of juvenile sablefish provided with different diets, and parameters of power regressions between daily weight gain (G) and weight (W) ($G = aW^b$).

Diet	N	mm/d	a	b	r^2
A10	24	0.35 (0.19)	—	—	—
A20	29	0.82 (0.13)	0.059	0.591	0.73
A30	32	1.25 (0.23)	0.085	0.761	0.95
AAd	10	2.00 (0.25)	0.134	0.702	0.94
M10	12	0.90 (0.13)	0.051	0.713	0.91
M20	15	1.52 (0.32)	0.086	0.851	0.94
M30	7	1.95 (0.30)	0.117	0.788	0.96
MAd	6	2.24 (0.22)	0.147	0.716	0.98
AAd[a]	11	2.18 (0.65)	—	—	—

[a]Preliminary trials in 1983; only length data were collected.

The maximum ration levels consumed daily by juvenile sablefish (≥30% bw/d) are larger than have been measured for juveniles of the few other species examined. Comparable laboratory studies that present daily consumption rates in similar units of percent wet body weight are summarized in Table 4. Consumption capabilities similar to sablefish were found only for neustonic larval and early juvenile billfish (Istiophoridae). Gorbunova and Lippskaya (1975) and Lippskaya and Gorbunova (1977) used stomach contents of field-preserved specimens to estimate daily consumption rates up to 30–45% bw/d.

Growth in fishes is often estimated from various sigmoid size versus age models (e.g. logistic and Gompertz curves, Ricker 1979). The slope (growth/time) of the central portion of the sigmoid curve is essentially constant. Growth of juvenile sablefish in 1984 on any diet (but A10) did not significantly change with increasing size, indicating that the 35- to 100-mm size range fits along the central portions of sigmoid growth curves. A possible exception to this constant daily growth was indicated in the preliminary trials in 1983. These small fish fed on an AAd diet had an initial size of 20–33 mm, with estimated growth during the first week averaging 3.0 mm/d. Growth during the following 2 wk decreased to a mean value of 1.7 mm/d, similar to that achieved by the AAd fish in 1984. The very rapid growth noted during the first week may indicate a growth pattern that maximizes growth in length over the 20- to 35-mm size range. Such a growth pattern may enhance the swimming ability of the small juveniles, which can aid in searching larger volumes of water for prey (Blaxter and Staines 1971) and in their ability to avoid predators (Parker 1971; Murphy and Clutter 1972). The measuring technique used in 1983 (visual estimation against a grid), however, was less precise than the process of anesthetization and direct measurement of the fish with calipers performed in 1984.

Our calculations of laboratory growth in juvenile sablefish closely approximated estimates of growth of wild specimens. Boehlert and Yoklavich (1985) developed a growth curve for wild larval and juvenile sablefish from length measurements and daily otolith ring increments. The resulting Laird–Gompertz growth model had a slope of 1.88 mm/d over the size range of 40–100 mm. This value is almost identical to the mean growth rate of fish in the same size range fed on a M30 diet (1.95 mm/d; Table 3), and slightly under the mean values of fish fed on AAd or MAd diets (2.00 and 2.24 mm/d, respectively). The similarity between the growth of wild juvenile sablefish and laboratory fish fed at high ration levels suggest that wild fish consume approximately 30% wet body weight/d.

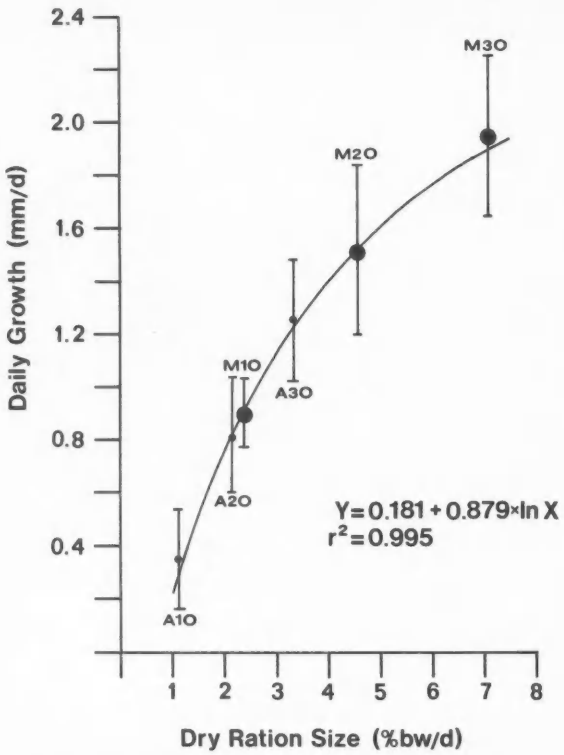

FIG. 2. Mean (SD) daily growth in length of sablefish achieved on dry weights of rations (dry weight of prey · 100%/wet weight of fish).

Our data indicate that juvenile sablefish possess the ability to grow more rapidly than juveniles of most other species (Table 5), although direct comparisons between studies can be misleading. Differences in growth among species may be due to variation in factors such as quantity and quality of food provided to the fish as well as different environmental conditions. In general, juveniles of demersal, coastal, and estuarine species grow relatively slowly, while several epipelagic fishes (*Coryphaena* spp., *Scomber scombrus*, *Katsuwonus pelamis*) have growth rates similar to or faster than the juvenile sablefish.

The allometric growth patterns achieved by sablefish fed on all but the lowest rations are indicated by length–weight power regression exponents > 3 (Table 2; Ricker 1979). These data show that the fish were increasing body robustness more rapidly than they were growing in length. However, isometric growth was noted in wild fish. The length–weight regression determined from 180 formalin-preserved specimens (31–79 mm) was $Wt = 9.04 \cdot 10^{-6} \cdot SL^{3.02}$ ($r^2 = 0.99$; J. M. Shenker, unpubl. data). Thus, although laboratory-reared fish fed at high ration levels grew in length at approximately the same rate as wild fish, the laboratory fish grew more rapidly in weight. The restricted activity of the fish in the aquaria appeared to be the most likely cause of the elevated weight gain.

The efficiency of the conversion of food into somatic tis-

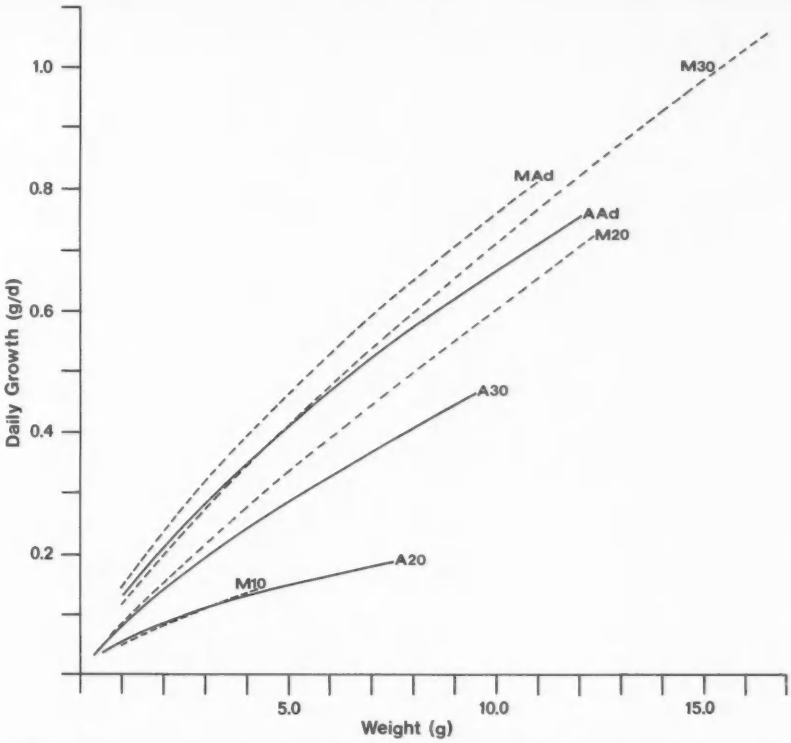

FIG. 3. Power regressions of daily weight gain against weight of sablefish fed different diets.

TABLE 4. Maximum food consumption rates of juvenile fishes (% wet body weight/d).

Species	Size	Temp. (°C)	Consumption (% bw/d)	Reference
Clupea harengus	7.5–11.8 g	6.5	3.5–5.0	De Silva and Balbontin 1974
	8.2–18.8 g	14.5	8.1–12.6	
Oncorhynchus keta	41–? mm FL (0.45–9.9 g)	14–16	17	LeBrasseur 1969
Urophycis chuss	92–157 mm TL	11	7.4	Luczkovich and Olla 1983
Sebastes melanops	63–69 mm SL	7	2.4[a]	Boehlert and Yoklavich 1983
	67–82 mm SL	12	5.0[a]	
	64–82 mm SL	18	6.6[a]	

[a]Per meal.

sue was markedly higher for mysid-fed fish than for *Artemia*-fed specimens (Fig. 4). Brett (1979) showed that the typical response of gross conversion efficiency to increasing ration size is a dome-shaped curve, with a gross conversion efficiency of 0 at very low ration levels, rising to a peak optimal efficiency at a higher ration (R_{opt}), and then decreasing to lower efficiencies at even higher rations. Data from both diet types indicate that similar curves may exist for the sablefish, but not enough ration sizes were tested to fully define the curves. For mysid-fed fish, R_{opt} was achieved at or near the lowest ration size. *Artemia*-fed fish had a shallower gross conversion efficiency curve with a R_{opt} in the vicinity of 20% bw/d. Again, the higher water content of the *Artemia* prey appeared to be the primary factor for the differences between the gross conversion efficiency curves for the two prey types. Without dry weight data for the fish, however, it was not possible to calculate gross conversion efficiencies on a dry weight basis.

The peak gross conversion efficiencies of approximately 40% determined for fish fed on mysids at low ration sizes are higher than have been observed for most other juveniles. *Sebastes melanops* was found to have gross conversion efficiencies reaching 20–22% (Boehlert and Yoklavich 1983),

TABLE 5. Maximum growth achieved by juvenile fishes receiving elevated rations in laboratory experiments, or in field studies. All lengths are SL unless otherwise specified. L = laboratory experiment; O = otolith aging study; C = cohort growth study; M = mark and recapture.

Species	Type of study	Ration size	Temp. (°C)	Length (mm)	Growth in length (mm/d)	Reference
Urophycis chuss	L	ad lib	11	92–157 TL	1.2	Luczkovich and Olla 1983
U. chuss	L	ad lib	6.7 11.5	50–177 TL 89–209 TL	0.6 1.5	Steiner et al. 1982
Hexagrammos otakii	L	?	10–17	20–45	0.5	Fukuhara and Fushimi 1983
Anoplopoma fimbria	L	ad lib	13	50–100	2.24	This study
Sebastes melanops	L	Satiation (once per day)	7 12 18	63–69 67–82 64–82	0.09 0.26 0.31	Boehlert and Yoklavich 1983
Mugil curema	C	—	20–31	25–142 FL	1.17–1.33	Richards and Castagna 1976
Micropogonias undulatus	M	—	—	25–86	0.5–1.0	Knudsen and Herke 1978
Coryphaena hippurus	O	—	—	60–400	4.7	Oxenford and Hunte 1983
Scomber scombrus	O	—	—	30–70	2.3	Kendall and Gordon 1981
Katsuwonus pelamis	O	—	—	40–270 FL	1.6	Uchiyama and Struhsaker 1981

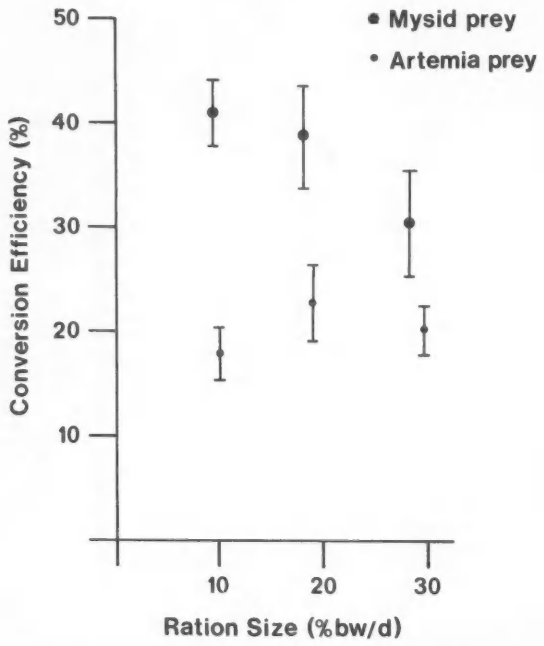

FIG. 4. Mean (SD) gross conversion efficiency of sablefish fed different diets.

while *Clupea harengus* was able to convert only 7–12% of the food weight into body weight (De Silva and Balbontin 1974). *Oncorhynchus nerka* had a maximum gross conversion efficiency of about 25%, at a R_{opt} of 4% bw/d (Brett et al. 1969).

The overall pattern of growth of neustonic juvenile sablefish indicated that these fish have the potential to grow very rapidly when sufficient food is present. Similar or faster growth rates were achieved only by several species whose juveniles also live in the epipelagic or neustonic realm. These planktivorous juveniles may have evolved under selective pressure to maximize their growth and rapidly increase their swimming ability. Such a growth strategy would enhance their ability to find individual food items or patches in the relatively dilute food environment of the open ocean, as well as aid in the avoidance of predators in an environment in which physical refugia are rare.

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Dietary Change during Development in the Freshwater Calanoid Copepod *Epischura lacustris* Forbes

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Chow-Fraser, P., and C. K. Wong. 1986. Dietary change during development in the freshwater calanoid copepod *Epischura lacustris* Forbes. Can. J. Fish. Aquat. Sci. 43: 938–944.

The diet of the freshwater calanoid copepod *Epischura lacustris* from C2 to adult stage was examined with predation experiments, grazing experiments, and stomach content analyses. There was a transition from an exclusively herbivorous diet in early copepodid stages to an omnivorous diet in adults. Laboratory predation experiments revealed that C2 and C3 were incapable of ingesting *Bosmina* (0.25–0.35 mm), but from C4 to C6, ingestion rate of *Bosmina* increased with developmental stage. The order or prey selection for adult *Epischura* was *Bosmina* over *Diaptomus* and *Cyclops*. Grazing rate on small algae (<10 μm) increased with developmental stage. Zooplankton remains were only found in the guts of stages older than C2; cladocerans and crustacean eggs were the most common zooplankton food. Algae with longest linear dimensions greater than 10 μm (e.g. *Sphaerocystis, Dinobryon*, and diatoms) were common in the guts of all developmental stages of *Epischura*. The occurrence of small unicells (<10 μm) in the guts decreased with developmental stage.

On a étudié le régime alimentaire du copépode d'eau douce *Epischura lacustris* du groupe des calanoïdes du stade C2 au stade adulte en faisant des expériences sur la prédation et sur le broutage et en faisant l'analyse des contenus stomacaux. On a noté le passage d'un régime exclusivement herbivore dans les premiers stades de copépodides à un régime omnivore chez les adultes. Des expériences sur la prédation en laboratoire ont révélé que les individus des stades C2 et C3 étaient incapables d'ingérer *Bosmina* (0,25 à 0,35 mm), mais que, en passant du stade de développement C4 à C6, le taux d'ingestion de *Bosmina* augmentait. Chez les adultes, les proies étaient par ordre d'importance *Bosmina*, puis *Diaptomus* et *Cyclops*. Le taux de broutage sur les petites algues (<10 μm) augmentait en passant d'un stade de développement à un autre. On n'a trouvé des restes de zooplancton que dans le tube digestif d'individus appartenant à des stades plus avancés que C2; les oeufs de cladocères et de crustacés constituaient les éléments du zooplancton le plus souvent ingérés. On retrouvait couramment dans le tube digestif des *Epischura* à tous les stades de développement des algues qui, dans leur plus grande longueur, mesuraient plus de 10 μm (p. ex. *Sphaerocystis, Dinobryon* et diatomées). Il y avait diminution du nombre de petits organismes unicellulaires (<10 μm) présents dans le tube digestif en passant d'un stade de développement à un autre.

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Epischura lacustris is one of the most common zooplankters in lakes of glaciated North America (Carter et al. 1980). The adult predatory feeding behavior of this calanoid copepod has been studied by many authors (Main 1961; Confer and Blades 1975; Strickler and Twombly 1975; Kerfoot 1977, 1978; Wong 1981a), especially with reference to its impact on the life history and morphology of the small cladoceran, *Bosmina* (Kerfoot 1975; Black 1980; Wong 1981b; Byron et al. 1984; Wong and Sprules 1985). Because of the emphasis on its predaceous feeding in these studies, investigators have tended to consider it only a carnivore in aquatic food webs (Sprules 1980; Sprules and Knoechel 1984), despite there being strong evidence that *Epischura* adults feed omnivorously on both algae and zooplankton prey (Main 1961; Chow-Fraser and Wong 1985; Wong and Chow-Fraser 1985). This biased view of its functional role is even more objectionable when we consider that the biomass of immature stages often surpasses that of adults throughout the year (Main 1961), and as yet, the predaceous feeding habit of immature instars has not been clearly demonstrated.

This tendency to consider only a portion of an animal's feeding habit is also evidenced in studies on other omnivorous calanoid copepods. For example, Folt and Goldman (1981) studied only the herbivorous feeding of *E. nevadensis* while Kerfoot (1978) concentrated only on its predaceous feeding. Kibby and Rigler (1973) reported only the grazing rates of *Limnocalanus macrurus* even though this calanoid copepod is an omnivore (Wong and Chow-Fraser 1985; Chow-Fraser and Wong 1985).

In comparison with studies on marine species (Allan et al. 1977; Fernandez 1979; Marshall and Orr 1956; Mullin and Brooks 1967; Paffenhöfer 1971, 1984), relatively little is known about diet changes that accompany the development of *E. lacustris*, or any other freshwater calanoid copepod. In this paper, we examine the dietary changes of *E. lacustris* from early copepodid to adult stages. We concentrate on both the

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TABLE 1. Description of study lakes. pH values are volume-weighted measurements from 1979 samples, except for Gull L. Data for Gull L. are taken from Ontario Ministry of Natural Resources, Minden, Ont. (unpubl. data).

Lake	Maximum depth (m)	Mean depth (m)	Volume (Mm^3)	pH
Gull	49.10	16.50	164.34	7.5
Mountain	31.39	13.43	44.12	6.5
Blue Chalk	21.95	9.37	4.70	6.0

predatory and herbivorous components of their feeding habit throughout their development from C2 to adult. We hope that such information will enable more accurate representation of all developmental stages of this calanoid copepod in future food web studies, so that studies of the energy budgets of plankton in lakes can be more realistic.

Methods and Materials

Three lakes were included in this study, Mountain, Blue Chalk, and Gull lakes, located in south-central Ontario. These lakes vary with respect to their basin morphology and water chemistry (Table 1) and were sampled at various times during 1981–84.

Predation Experiments

Epischura for predation experiments were collected from the epilimnion of Blue Chalk L. (May and June 1984) and Gull L. (August 1983). These samples included all developmental stages from C2 to adults. Prey for these experiments included *Bosmina longirostris* (0.25–0.35 mm, from the rostrum to base of the mucro) from laboratory cultures and adult stages of *Cyclops* spp. (0.8 mm, body length) and *Diaptomus minutus* (0.7 to 1.0 mm, body length) collected from Gull L.

For experiments with *Bosmina*, one predator was introduced into 200 mL of 64-μm-filtered lake water containing five prey individuals. We conducted 2, 4, 20, 12, and 12 experiments for each of the instars of *Epischura* ranging from C2 to C6, respectively. We also conducted 4, 3, and 2 experiments with adult *Epischura* using *Cyclops* as prey, at densities of 5, 10, and 15 prey·L^{-1}, respectively; we similarly conducted 5, 3, and 4 experiments using adult *Diaptomus* as prey at densities of 5, 10, and 15 prey ·L^{-1}, respectively. In these predation experiments, one to three predators were added to beakers containing 1 L of 64-μm-filtered lake water.

Predation durations, ranging from 6 h (for *Bosmina*) to 12 h (for *Cyclops* and *Diaptomus*), were chosen so that some prey were still alive at the end of the experiments. All experiments were carried out at 10–15°C in total darkness. At the end of the experiments, all remaining animals were retained on a small 64-μm Nitex screen and examined with a stereomicroscope. Since no prey mortality was found in control containers (beakers with identical contents except predators), predation rates were determined from the difference between the initial number of prey and the number of prey found alive at the end of the experiments.

Grazing Experiments

In situ grazing experiments were conducted in Mountain L. in July 1981, Blue Chalk L. in June 1982, and Gull L. in June and August 1982. All experiments were conducted in the epilimnion; 5 experiments were conducted in Blue Chalk L., 11 in Gull L., and 2 in Mountain L.

We used the procedure of (Haney 1971) for in situ experiments. Animals were collected within the water column with a 2-L Haney grazing chamber. They were fed ^{14}C-labelled *Chlorella vulgaris* (in 1981), *Scenedesmus ovalis* (in 1982), and *Pediastrum* spp. (in 1982). The longest linear dimensions of these algal particles were 6–8 μm for *Chlorella*, 4–5 μm for *Scenedesmus*, and 30–40 μm for *Pediastrum* (coenobium). Algae were prepared for radioactive labelling according to Holtby and Knoechel's (1981) purge–uptake method. The specific activity of water in the grazing chamber generally ranged from 1000 to 5000 cpm·mL^{-1}. Animals grazed in situ for 15 min. They were then filtered onto modified petri plates equipped with 64-μm Nitex screens and killed by immersion into hot water (at least 80°C). They were rinsed with filtered (64 μm) surface lake water and then stored on dry ice for no longer than 7 d. To determine the amount of radioactivity in the grazing chamber, 5-mL aliquots of the grazing chamber water were collected.

In the laboratory, animals were sorted and identified with the aid of a dissecting microscope. We measured the prosome length for each animal and then placed each into individual scintillation vials. Animals were digested overnite in 0.25 mL of NCS (Amersham) tissue solubilizer at 50°C. We then followed Chow-Fraser and Knoechel's (1985) method for scintillation counting. Radioactivity in the animals ranged from 3 to 100 counts above background, but were usually between 30 and 60 cpm·$animal^{-1}$.

The prosome length of 20 individuals of each developmental stage was measured from preserved zooplankton samples (70% alcohol) from Blue Chalk L. The range of these measurements were used as an index for estimating the developmental stage of *Epischura* in the in situ grazing experiments, since only prosome lengths were recorded.

Laboratory grazing experiments were conducted with animals from Gull L. (August 1982). Five adults were placed in 1 L of 64-μm-filtered lake water and were acclimatized in beakers at 10°C for 2 h prior to experimentation. One millilitre of labelled *Scenedesmus* or *Pediastrum* was then introduced into the beaker and gently mixed with a glass rod. Animals were allowed to graze for 30 min and were then killed with hot water, rinsed, sized, and placed individually into scintillation vials. Other details of the procedure were essentially as already described. In these experiments, radioactivity in each animal ranged from 5 to 150 counts above background.

Stomach Content Analysis

Animals for stomach content analyses were collected from Blue Chalk L. during June 1984 by vertical tows of the epilimnion. They were stored in Lugol's iodine solution to preserve the algal and zooplankton material in the guts.

The prosome length and developmental stage were recorded for each animal. Stomachs were dissected out with fine pins with the aid of a dissecting microscope. They were then transferred onto a slide and gently squashed under a glass coverslip, with addition of a dilute glycerin–water solution. Contents were examined at 200× and 400× magnification using a phase-contrast light microscope.

The results of stomach content analyses are dependent on the availability of food items in the water column, the different digestibility of the various food types, the condition of the stomach dissected out for examination, and the correctness of

the stomach content identification. Since we anticipated that experience would enable a more thorough identification of later relative to early specimens, we examined developmental stages in random order.

Statistical Analyses

Grazing rates of *Epischura* (all developmental stages) were regressed against prosome length, regardless of lake origin, with a least-squares regression analysis (Sokal and Rohlf 1981). Lake-by-lake regression equations were also generated and analysis of covariance (Sokal and Rohlf 1981) was used to determine differences among slopes and intercepts of these lakes. Nonparametric analyses included the Kruskal–Wallis test and the Mann–Whitney *U*-test (Zar 1984).

Results and Discussion

Predation Experiments

Generally, adult *Epischura* had very low predation rates on adult *Cyclops* and *Diaptomus*, the maximum mean ingestion rate being only 0.5 prey·predator^{-1}·d^{-1}. They did not have measurable ingestion rates when presented with adult *Cyclops* at prey densities of 5 or 15 prey·L^{-1}; at a prey density of 10·L^{-1}, there was a mean predation rate of 0.4 prey·predator^{-1}·d^{-1}. *Epischura* adults appeared to have higher ingestion rates when presented with adult *Diaptomus;* at prey densities of 5 and 15 prey·L^{-1}, they had a mean ingestion rate of 0.2 and 0.5 prey·predator^{-1}·d^{-1}, respectively, although no measurable ingestion rate was recorded at a prey density of 10·L^{-1}. We did not attempt to conduct predation experiments with immature stages of *Epischura* using these two prey species, since predation rates would likely be too low to measure.

By comparison, adult *Epischura* from Blue Chalk L. had high predation rates on *Bosmina*. We found that predation rate on *Bosmina* increased with developmental stage (Kruskal–Wallis test, $P < 0.05$), from a mean rate of 3 prey·predator^{-1}·d^{-1} at C4 to 6 prey·predator^{-1}·d^{-1} at C6 (Fig. 1). Predation rates of C5 and C6 were statistically similar (Mann–Whitney *U*-test, $P > 0.05$), but those of C4 were statistically lower than C5 and C6 combined (Mann–Whitney *U*-test, $P < 0.01$), suggesting that predation rate is proportional to predator size. There was, however, no measurable ingestion rate on *Bosmina* for C2 and C3 stages.

Individual variation in daily predation rate of *Bosmina* was high. Such high variability in predation rate may be the result of intermittent feeding behavior of *Epischura*, and also reflects the difficulty associated with successful capture and ingestion of *Bosmina* by this predator (Kerfoot 1978; Wong 1981b).

Main (1961) conducted predation experiments with *E. lacustris* using *Bosmina coregoni* as prey but concluded that *Bosmina* was not a suitable prey for *Epischura*, since he obtained extremely low predation rates. The reason for his low rates relative to ours is probably because he used prey >0.5 mm, which were likely too big to be ingested by *E. lacustris* (Wong 1981a; Wong and Sprules 1985). Main also obtained results different from ours in that he measured high predation rates for *Epischura* feeding on *Diaptomus*. We believe that the probable reason for this discrepancy is that Main conducted predation experiments in 2-mL depression chamber slides. Since *Diaptomus* defends itself by outswimming the predator, experiments conducted in such unrealistically small volumes would artificially inflate predation rates for

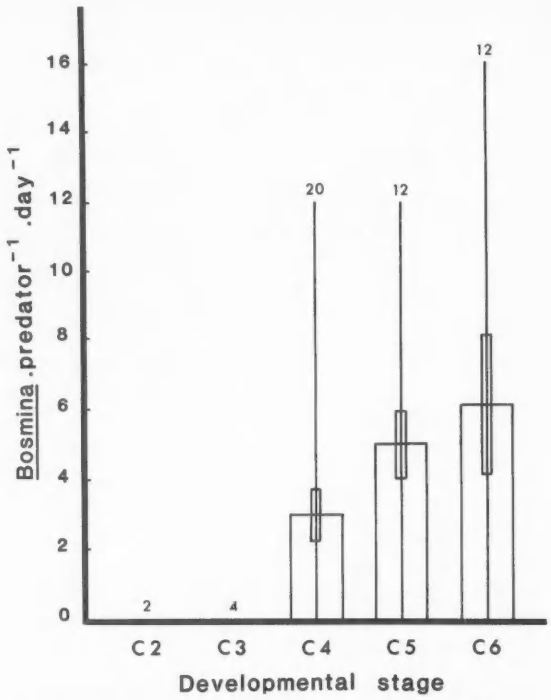

FIG. 1. Predation rate (mean ± 1 SE) by *Epischura lacustris* of various developmental stages on *Bosmina longirostris*. Numbers on top of bars indicate sample size.

Diaptomus. In another study, Folt and Goldman (1981) observed that *E. nevadensis* adults ingested adult *Diaptomus* at high rates in the laboratory, but did not have high predation rates in the field. Thus, *Epischura* probably do not prey on adult *Diaptomus* at high rates in nature, and this is supported by our data.

Grazing Experiments

In total, 61 in situ individual grazing rates were measured in the three lakes; 21 were from Blue Chalk, 27 from Gull, and 13 from Mountain L. The grazing rate when *Chlorella* was used as tracer declined logarithmically with body size (Fig. 2). Since we were unable to identify the developmental stage of the animals, we present the size range for each of the copepodid stage (Blue Chalk L.) below the *x*-coordinate in Fig. 2. As in the predation experiments, no C1 and relatively few C2 were present in samples because grazing data were collected in all three lakes between June and August when C1 and C2 were scarce relative to other stages.

The negative slope of the regression (Fig. 2) indicates that *Epischura* decreases its grazing on small algae such as *Chlorella* as it grows from C2 to adult. Consequently, the feeding of adult animals on such small cells is almost negligible. These low grazing rates on small algae were also reported in other omnivorous calanoid copepods (Kibby and Rigler 1973; Chow-Fraser and Wong 1985).

There is considerable individual variation associated with grazing rates of a single body size. Such high variability is not uncommon for field animals, and is not associated with differences in tracer cells used, since there was very high variability

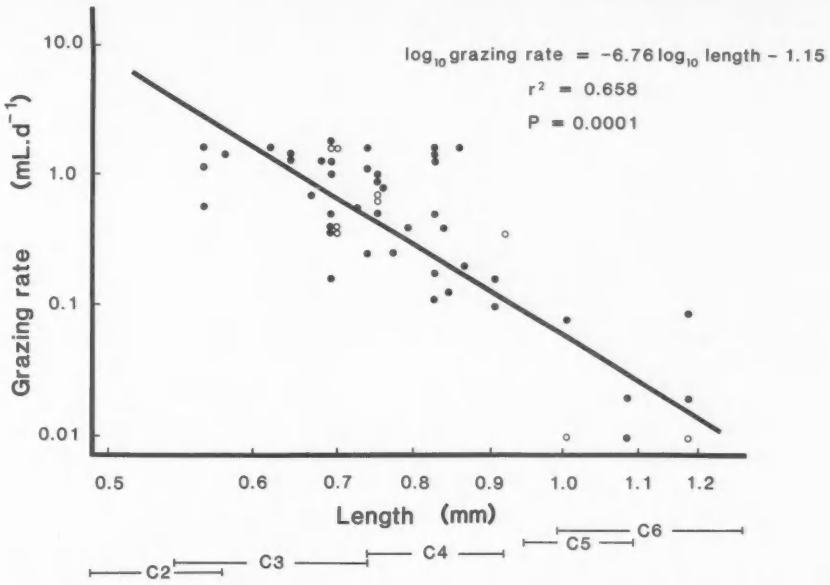

FIG. 2. Grazing rate versus prosome length for *Epischura lacustris*. Tracer cells used were *Chlorella*. Solid line represents least-squares regression through data. Two overlapping data points are represented by open circles.

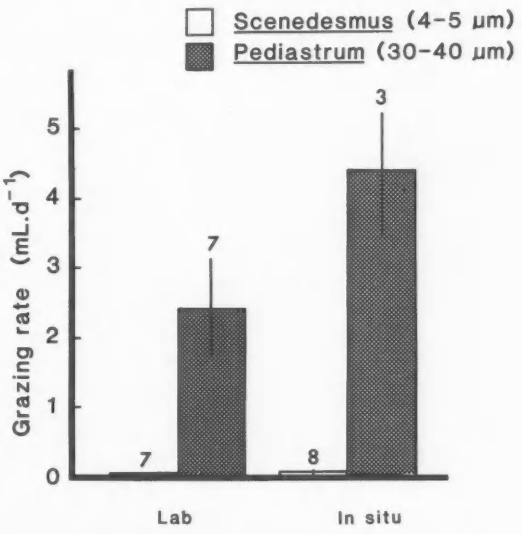

FIG. 3. In situ and laboratory grazing rate (mean ± 1 SE) of adult *Epischura lacustris*. Tracer cells used were *Scenedesmus* and *Pediastrum*. Numbers on top of bars indicate sample size.

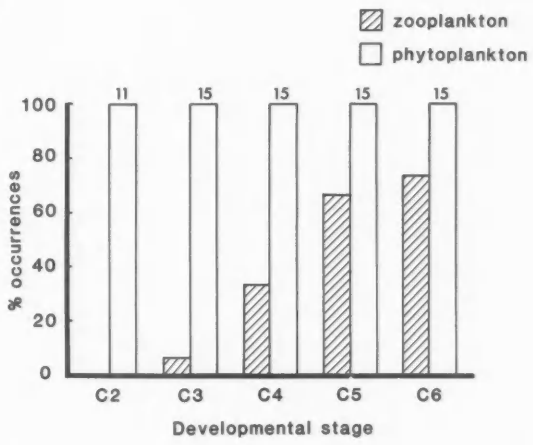

FIG. 4. Percent occurrence of zooplankton and phytoplankton in the guts of *Epischura lacustris* of various developmental stages.

even for data corresponding to one tracer species. Some of the variation may be attributed to the overlapping size of developmental stages; however, the highest individual variation was associated with C4 where there was no overlap in size with other stages. Comparison of regression equations for each lake (analysis of covariance) indicated that sopes and intercepts were statistically homogeneous ($P > 0.05$). Therefore, differences in lakes did not have any significant effect on the grazing rates of animals in the overall regression analysis.

Epischura adults grazed at higher rates on *Pediastrum* than on *Scenedesmus* in the laboratory and field studies (Mann–Whitney, $P < 0.025$ for both; Fig. 3). Grazing rates were generally less than 0.05 mL·d^{-1} when *Scenedesmus* was used, compared with mean rates of 2.43 and 4.36 mL·d^{-1} in laboratory and field experiments, respectively, when *Pediastrum* was used.

Stomach Content Analysis

Results of stomach content analyses (Fig. 4) substantiated all findings of the predation and grazing experiments (Fig. 1 and 2, respectively). We found algal remains in all of the animals regardless of developmental stage; however, the percent occur-

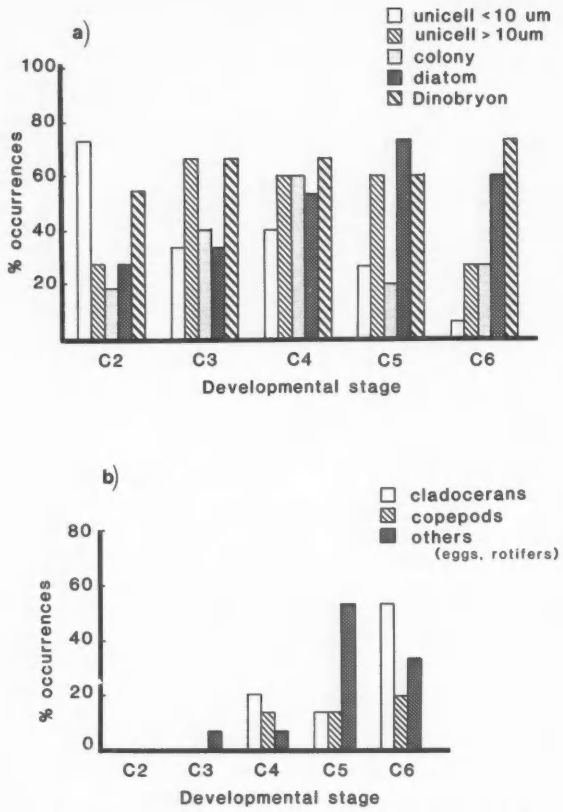

FIG. 5. Percent occurrence of (a) zooplankton and (b) phytoplankton in guts of *Epischura lacustris* of various developmental stages.

rence of animal remains increased with developmental stage, from 0% occurrence in C2 guts to 70% in adults.

Animal remains chiefly included cladocerans and copepods, although a large number of crustacean eggs were also present in the later instars (Fig. 5a). Adults were most predaceous on cladocerans, presumably *Bosmina*, but instars younger than C4 did not ingest any cladocerans. Since *Epischura* does not ingest the carapace of *Bosmina*, our estimate of the percent occurrence of cladocerans in these guts is probably conservative because we were not always able to distinguish the soft tissues of *Bosmina* from the mush present in the stomachs. The relative occurrence of copepod parts did not exceed 25% in any developmental stage, and substantiates the low predation rate of *Epischura* on *Diaptomus* and cyclopoids.

We categorized the algal remains into five groups: unicells <10 μm, unicells >10 μm, colonial forms, diatoms, and *Dinobryon* cells or colonies (Fig. 5b). By far, the most common food item in all developmental stages was *Dinobryon*. It occurred in at least 55% of guts of all stages. Small unicells were most common in C2, and became progressively rarer in the older stages; large cells, by contrast, were most common in the intermediate stages (C3 to C5), and relatively unimportant in C2 and C6. The dominant diatom was *Cyclotella*, and this was present in a large percentage of guts in the older instars. Colonial forms, especially *Sphaerocystis* and *Coelosphaerium*, were commin in C3 and C4, but were relatively uncommon in the stomachs of older or younger stages.

TABLE 2. Relative abundance of zooplankton in Blue Chalk L. (data from M. Arts, Department of Zoology, University of Toronto.) Samples were collected with a vertical townet from sediment to lake surface (mesh size 130 μm; mouth area 1590 cm^2; 70% efficiency)

Sampling date	Zooplankton taxa	Density (no.·L^{-1})	Size range (mm)	Mean size (mm)
3 June 1982	Nauplii	16.69	0.12–0.39	0.23
	Daphnia	3.29	0.62–1.88	1.17
	Holopedium	0.65	0.62–2.00	1.31
	Bosmina	2.49	0.21–0.74	0.42
	Diaptomus	29.07	0.23–1.00	0.62
	Cyclopoids	28.84	0.32–1.47	0.69
17 June 1982	Nauplii	16.05	0.11–0.53	0.22
	Daphnia	6.96	0.42–2.49	1.47
	Holopedium	0.22	1.22–2.53	1.80
	Bosmina	9.69	0.27–0.73	0.47
	Diaptomus	44.81	0.32–1.22	0.65
	Cyclopoids	65.10	0.35–1.48	0.63

The low percent occurrence of small cells in C6 is consistent with the observation that adult *E. lacustris* grazed on *Scenedesmus* and *Chlorella* at low rates. The relatively large contribution of colonial forms, diatoms, and *Dinobryon* in the gut contents of C2 and C3 precludes the notion that early instars are incapable of ingestion large phytoplankton. Immature *E. lacustris* ingests a large variety and size of algae; adults, on the other hand, mostly ingest large cells.

Main (1961) likewise observed greater than 30% occurrence of colonial algae in the stomachs of C5 and C6 animals. Diatoms such as *Synedra* and *Navicula* were commonly ingested (15–20%); however, *Dinobryon* were absent, even though they were present in the water column. The difference between his results and ours is perplexing, since *Dinobryon* were commonly found in the guts of other calanoid omnivores, *E. lacustris*, and *Senecella calanoides* in Gull L. (Chow-Fraser and Wong 1985). We speculate that its absence in the stomachs of Main's animals probably reflects the relative scarcity of *Dinobryon* in his lake at the time of sampling.

We can give a qualitative evaluation of *Epischura*'s food preference, since we noted the relative abundance of zooplankton and phytoplankton in Blue Chalk L. at roughly the same time of the season when samples were collected for the stomach content analysis. The most common prey items in the lake on both June sampling dates were diaptomids and cyclopoids (Table 2). Because cladocerans were less abundant than copepods in the water column, but were found in disproportionately high numbers in guts, we suggest that *Epischura* selectively feeds on cladocerans, presumably *Bosmina*. This trend of selective feeding is probably stronger if we consider that assessment of cladocerans in our analyses is conservative.

The dominant algae in Blue Chalk L. were forms larger than 30 μm. *Dinobryon* colonies, as well as diatoms such as *Tabellaria*, *Cyclotella*, and *Asterionella*, were prevalent (Table 3). Thus, it is not surprising that these algae were found in abundance in the stomachs of *Epischura*. *Cryptomonas* was also relatively abundant, but these were seldom in guts. Since these algae are naked, even if they had been ingested, they may have been macerated beyond recognition. It is surprising, though, that colonial forms such as *Sphaerocystis* and *Aphanocapsa* were present in guts, since these were relatively scarce in the water column. Thus, *E. lacustris* appears to select for these

TABLE 3. Biomass concentration of phytoplankton in Blue Chalk L. Sample was collected on 10 June 1982 from the epilimnion of the lake.

Phytoplankton taxa	Biomass ($mg \cdot m^{-3}$)
Colonial	
Sphaerocystis	25.67
Aphanocapsa	8.65
Chroococcus	12.48
Oocystis	9.01
Unicells	
Cryptomonas	48.54
Chlorella	32.13
Mallomonas	36.56
Diatoms	
Asterionella	54.84
Tabellaria	63.31
Cyclotella	170.36
Others	
Dinobryon	395.57

colonial forms in Blue Chalk L. This is also consistent with the observation that *Epischura* appeared to select for *Sphaerocystis* in Gull L. (Chow-Fraser and Wong 1985).

General Discussion

This study represents one of the few studies on the diet changes that accompany development of an omnivorous freshwater calanoid copepod. The results of two other studies (Elster 1936; Maly and Maly 1974) are consistent with the present findings. Elster (1936) studied the diet of *Heterocope borealis*, noting that the intensity of carnivorous feeding increased during copeodid development, and that all stages included a large quantity of *Cyclotella*, *Asterionella*, and *Synedra* in their guts. Maly and Maly (1974) noted a shift to larger algal particles with development of *Diaptomus shoshone* from C2 to adult; at C4, they became predatory, and thereafter maintained an omnivorous diet. It appears that the diet of freshwater omnivorous calanoids develops from soley herbivorous to completely omnivorous, with the transition to predaceous feeding at C4. Further investigation should be conducted to determine if the change to omnivory in C4 is accompanied by the development of sharp mandibular teeth in *Epischura*, similar to that noted by Maly and Maly (1974) for *D. shoshone*.

Proper evaluation of an animal's status in the food web requires detailed information on its feeding ecology. In this study, we have examined changes in ingestion rates of *E. lacustris* on various prey items during development. By examining rate changes, we have presented a more complete picture of the feeding of *Epischura* and have shown that C2 to C6 instars exert a differential impact on the phytoplankton and zooplankton prey in the water column. Future dietary studies of omnivorous copepods should include both the predatory and herbivorous components of their feeding habit so that the relative proportion of time spent on each feeding mode and the energetic consequences of switching food habit can be investigated.

Acknowledgments

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Experimental Infection and Pathogenesis of Viral Erythrocytic Necrosis (VEN) in Atlantic Cod, *Gadus morhua*

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Mature Atlantic cod (*Gadus morhua*) were experimentally infected with viral erythrocytic necrosis (VEN) by inoculation with washed erythrocytes or cell-free homogenates of erythrocytes from naturally infected fish. Approximately one third of the animals exposed exhibited active infections. The temporal pattern of infection was similar between naturally infected and experimentally infected fish. One to two months after infection, immature erythrocytes began to show clear evidence of VEN followed by a rapid increase in the proportion of infected immature erythrocytes, frequently reaching 100%. A subsequent dramatic drop in infection of immature erythrocytes occurred, coinciding with an increase of infection in mature erythrocytes. Significant erythroblastosis occurred when the overall erythrocyte infection rate reached approximately 10%, but none of the newly generated erythrocytes appeared infected. The peak infection rate (40–60% of erythrocytes infected) declined slowly and the infection, in most instances, was completely resolved.

On a infecté expérimentalement des morues de l'Atlantique (*Gadus morhua*) adultes par la nécrose virale des globules rouges (NVGR) en leur inoculant des érythrocytes lavés ou des broyats d'érythrocytes exempts de cellules provenant de poissons contaminés naturellement. Environ un tiers des poissons exposés ont montré des signes d'infection. Le mode d'infection dans le temps était semblable pour les poissons contaminés expérimentalement et pour ceux qui l'étaient naturellement. Entre un et deux mois après l'infection, les érythrocytes immatures ont commencé à montrer des signes évidents de NVGR et il y a eu subséquemment une augmentation rapide de la proportion d'érythrocytes immatures infectés, qui atteignait souvent 100 %. Il y a eu par la suite une baisse considérable d'érythrocytes immatures infectés, qui coincidait avec une augmentation du nombre d'érythrocytes matures infectés. Il y a eu une importante érythroblastose lorsque le taux global d'infection des érythrocytes a atteint environ 10 %, mais aucun des érythrocytes nouvellement formés n'a semblé contaminé. Il y a eu une baisse lente du taux maximal d'infection (40 à 60 % des érythrocytes infectés) et l'infection, dans la plupart des cas, s'est complètement résorbée.

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Viral erythrocytic necrosis (VEN) has been identified in a wide range of poikilothermic vertebrates including fishes (Appy et al. 1976; Doyle 1971; Evelyn and Traxler 1978; Johnston and Davies 1973; Laird and Bullock 1969; Reno et al. 1978), amphibians (Bernard et al. 1968), and reptiles (Stehbens and Johnston 1966). The infection is characterized by the presence of cytoplasmic inclusion bodies visible by light microscopy in infected erythrocytes. Examination by electron microscopy reveals that these inclusions are associated with one of several types of large (150–360 nm) icosohedral deoxyriboviruses provisionally classified as members of the *Iridoviridae* (Appy et al. 1976; Evelyn and Traxler 1978; Reno et al. 1978; Smail and Egglestone 1980). Whereas all erythrocytic necrosis viruses (ENV) associated with VEN in a variety of Pacific Ocean fish species appear identical in size and ultrastructure (Evelyn and Traxler 1978; MacMillan and Mulcahy 1979), several apparently species-specific ENV that differ markedly in size and ultrastructure have been described in Atlantic Ocean fishes (Appey et al. 1976; Johnston and Davies 1973; Reno et al. 1978).

In vitro propagation of ENV has thus far been unsuccessful (Evelyn and Traxler 1978; Reno and Nicholson 1980). However, ENV of Pacific salmonids (*Oncorhyncus* sp.) and herring (*Clupea harengus pallesi*) has been successfully transmitted in vivo to juvenile salmon (Evelyn and Traxler 1978; MacMillan and Mulcahy 1979; MacMillan et al. 1980). These studies demonstrated that VEN infection in Pacific salmonids did not cause mortalities directly but did result in severe anemia and increased susceptibility to secondary bacterial infections and

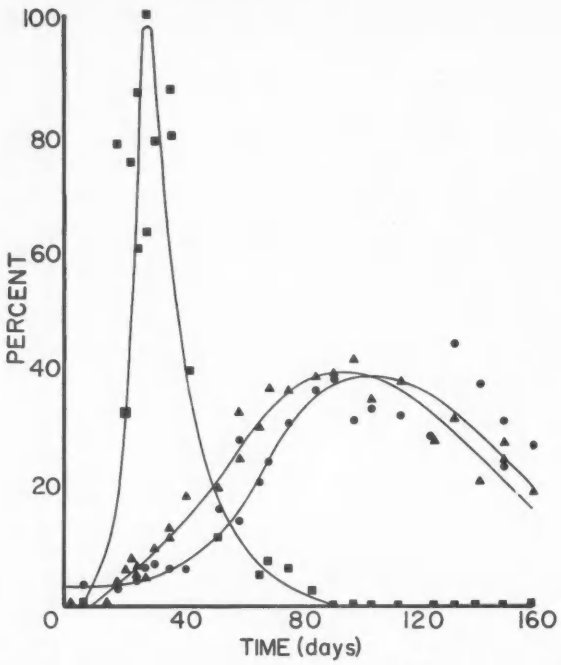

FIG. 1. Time course of natural VEN in adult Atlantic cod. Initial infection rate 0.01%. ■, VEN-infected immature erythrocytes; ▲, total erythrocytes infected; ●, uninfected, immature erythrocytes.

adverse environmental effects. It has recently been demonstrated that VEN infection of Atlantic cod (*Gadus morhua*) and herring (*Clupea harengus harengus*) results in a moderate normocytic anemia and alteration of erythrocyte metabolism (Reno et al. 1985).

Although ENV infection in most affected species has been observed in erythrocytes in all stages of development, it is not known which cells are initially infected and little information is available about the time course and pathogenesis of the infection.

The objective of this study was to determine if cod ENV could be transmitted to adult Atlantic cod and, if transmission was accomplished, to trace the development of the infection in comparison with that in naturally infected fish. This would lead to a better understanding of the disease process in cod and help assess the potential effect of the disease on wild populations.

Materials and Methods

For most studies on the experimental transmission of ENV, mature Atlantic cod, ranging in size from 30 to 50 cm total length, were collected over a 2-yr period by drag net near Boothbay Harbor, Maine. They were held for 2–4 wk prior to use in 3-m-diameter circular plastic pools supplied with untreated, running seawater at ambient temperatures of 2–7°C. For most infection experiments, fish were transferred to 2-m-diameter pools supplied with recirculating artificial seawater (Instant Ocean, Fisher Sci., Medford, MA) at temperatures of 5–10°C. Fish were fed smelt sterilized by autoclaving. For more detailed studies of the time course and pathogenesis of disease, mature cod were collected as before and held in quarantine for 2 wk in 3-m plastic pools supplied with running, untreated seawater. After the quarantine period, the fish were housed in the same pools and used for infectivity studies.

TABLE 1. Experimental transfer of erythrocytic necrosis virus (ENV) infection in cod.

Exp. No.	Infection rate of donor (%)	Preparation of inoculum[a]	VEN positive/number exposed[b]	% infection
1	2	Washed E	0/15	0
	0	Washed E	0/5	0
2	45	Washed E	3/13	23
	0	Washed E	0/7	0
3	16	E lysate	11/14	78
	0	E lysate	0/3	0
4	8	E lysate	4/13	30
	0	E lysate	0/7	0
Total			21/63	33

[a]E = erythrocytes.
[b]Recipient fish were tested for VEN at least three times over a period of several months.

Over 2 yr, several attempts were made to transmit ENV to adult cod. Early studies were designed to determine if the virus could indeed be transmitted, while later attempts were made to trace the development of disease. Blood was collected by caudal vascular puncture from naturally infected cod as a source of virus for experimental transmission studies. Six donor fish were used as a source of ENV during the course of four attempts to transmit the virus to a total of over 60 fish. Blood from apparently uninfected fish was similarly collected for mock infection of 23 fish. Blood cells were washed three times by centrifugation at 50 × *g* in marine phosphate buffered saline (MPBS) (Reno and Nicholson 1980) and the cell pellet was resuspended in four volumes of MPBS. Fish to be used for infectivity studies were held 2–4 wk before use and then injected either intraperitoneally (0.4 mL) or intravascularly (0.2 mL) with washed whole blood cells or cell-free erythrocyte lysates prepared by hypotonic lysis.

Evidence for VEN infection was obtained by collecting blood from the efferent branchial artery or caudal puncture, preparing thin blood smears, staining with Giemsa, and examining by light microscopy as described previously (Reno et al. 1978). The erythrocyte infection rate was expressed as the percentage of VEN-infected erythrocytes per 500–600 erythrocytes.

For a more detailed study of the initiation, progression, and pathogenesis of VEN, a single study was performed in which 14 cod were experimentally or mock infected or not injected and the blood sampled from each fish at weekly intervals for a period of 6 mo to evaluate both the VEN status and the numbers of immature erythrocytes in the peripheral circulation. Since there apparently are no recorded descriptions of the erythropoietic series in Atlantic cod, immature erythrocytes were classified on the basis of their morphological similarities to those of other fishes (MacMillan and Mulcahy 1979; Sherburne 1973). Nonmyelogenous cells which were morphologically distinguishable from mature erythrocytes, generally on the basis of cytoplasmic tint and shape, were considered to be erythrocyte precursors.

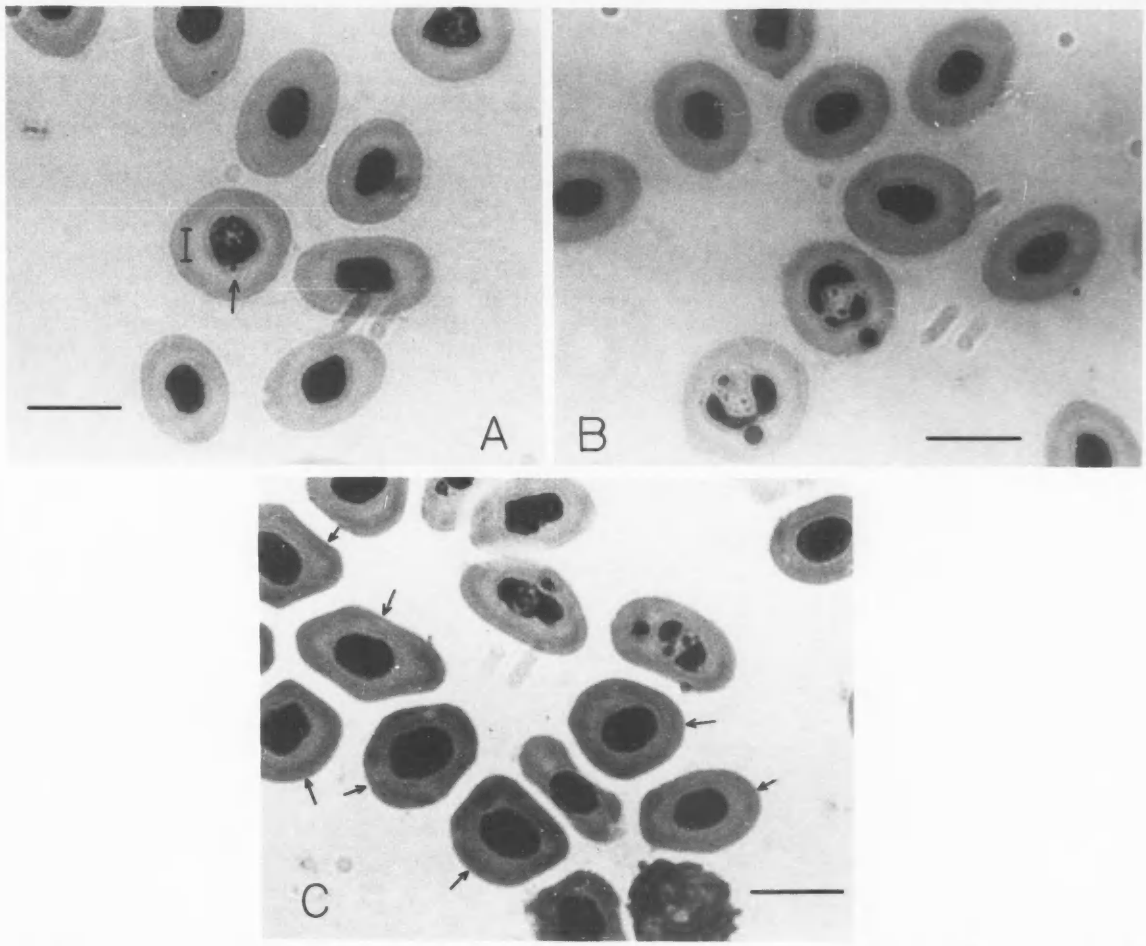

FIG. 2. Progress of experimental VEN infection in adult Atlantic cod. (A) 15 d after exposure. Immature erythrocyte (I) with characteristic rounded shape and round nucleus exhibiting early effects of VEN: chromatin margination and coalescence and a small, round perinuclear inclusion body (arrow). (B) 50 d after exposure. Two immature erythrocytes showing VEN inclusions and severe karyorrhexis. No evidence of VEN in mature erythrocytes. (C) 109 d after exposure. Approximately 40% of the erythrocytes show evidence of severe nuclear degeneration characteristic of VEN. Marked erythroblastosis has occurred, but none of the immature erythrocytes (arrows) appear to be infected. Bar represents 10 μm.

Tissues from kidney, spleen, liver, heart, intestine, and gonads were fixed in MPBS-buffered formalin (10%), embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin.

Results

Although during the course of these studies nearly 100 VEN-infected Atlantic cod were obtained and followed through the clinical phase of VEN on an individual basis, only infrequently were fish found to be in the initial phase of infection. The data in Fig. 1 represent the course of infection of one such animal; approximately 20 others monitored during later phases of the disease followed approximately the same temporal pattern.

When first examined, the erythrocytes of the fish depicted in Fig. 1 had an infection rate of less than 0.01%. This rate gradually increased and peaked after 3 mo of captivity at approximately 40% of the erythrocytes being necrotic; a gradual decline in the erythrocyte infection rate then ensued until about half of the peak value was reached at the termination of the experiment at 160 d. Nearly 20 other individuals examined during the recovery phase of the infection were able to clear peripheral blood of necrotic erythrocytes after being held for several months.

During the course of the infection, a marked erythroblastosis occurred, commencing when the erythrocyte infection rate reached between 5 and 10%; erythroblastosis peaked the same time as VEN infection rate and declined at approximately the same rate as erythrocyte infection. The shape of this curve was similar to infection curves generated for other infected individuals, and generally, there was a correlation between VEN infection rate and the proportion of immature erythrocytes.

The most striking finding, however, was the course of infection in immature erythrocytes. These were the first cells

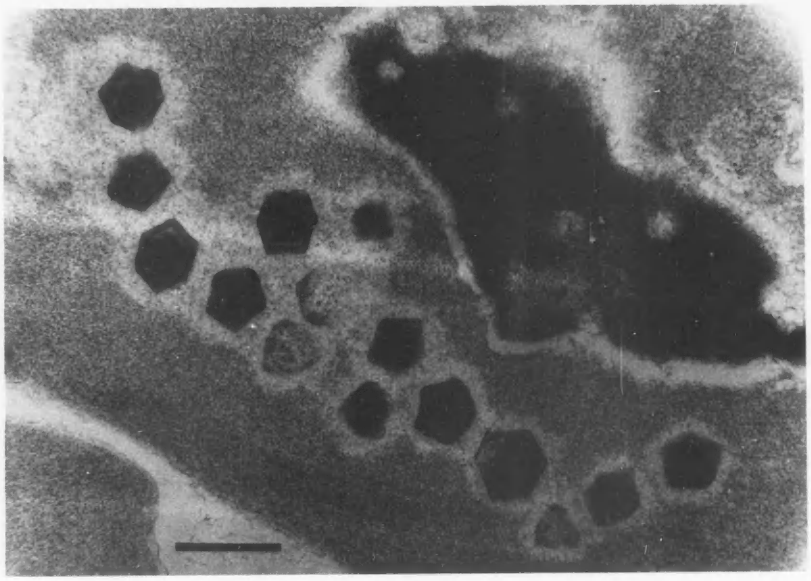

FIG. 3. Electron micrograph of erythrocytes from experimentally infected adult cod 90 d after exposure. Numerous hexagonal and pentagonal particles characteristic of ENV are seen in the cytoplasm. Bar represents 500 nm.

TABLE 2. Development of VEN in individual experimentally infected cod.

Source of blood for inoculum	% ENV in inoculum	Route of inoculation[a]	Outcome of inoculation[b]	Days to appearance of VEN	Maximum infection rate (% VEN positive cells)	Days to maximum level of VEN
ENV+	4.5	i.p.	+	39	22	142
ENV+	4.5	i.p.	+	25	40	99
ENV+	4.5	i.v.	−	—	—	—
ENV+	21	i.p.	+	32	32	≥90
ENV−[c]	0	i.p.	−	—	—	—
ENV−[c]	0	i.p.	−	—	—	—
ENV−[c]	0	i.v.	−	—	—	—
ENV−[d]	0	i.p.	−	—	—	—
ENV−[d]	0	i.p.	−	—	—	—
ENV−[d]	0	i.p.	−	—	—	—
ENV−[d]	0	i.v.	+	7	48	142
Not injected			−	—	—	—
Not injected			−	—	—	—
Not injected			−	—	—	—

[a]i.p. = intraperitoneal; i.v. = intravenous.
[b]After exposure, fish were examined weekly for 90−142 d.
[c]Determined to be ENV-positive during quarantine, but ENV-negative at time of experiment.
[d]Mock infection.

infected and showed evidence of necrosis much more rapidly and to a higher degree than did mature erythrocytes; in addition, the decline in infection rate of immature erythrocytes was also precipitous, with the majority of the infected immature cells occurring within the first month. Peak infection in immature erythrocytes, in this case 100%, was coincident with erythroblastosis.

Similar patterns of infection have been observed in about 20 other naturally infected cod sampled sequentially. Quantitative differences were noted primarily in the maximal infection rate, which varied between 10 and 65%; the basic shape of the erythrocyte infection curve, however, varied little.

At no time during these experiments did any individuals show any gross or histopathological evidence of disease in visceral organs including kidney, spleen, liver, heart, intestine, and gonads.

In addition to monitoring the development of VEN in feral cod, the ability to experimentally infect fish with ENV was evaluated in order to provide a system for more detailed investigations of the pathogenesis of the infection. The results of experimental and mock infection of several groups of cod over a 2-yr period are shown in Table 1. The success of experi-

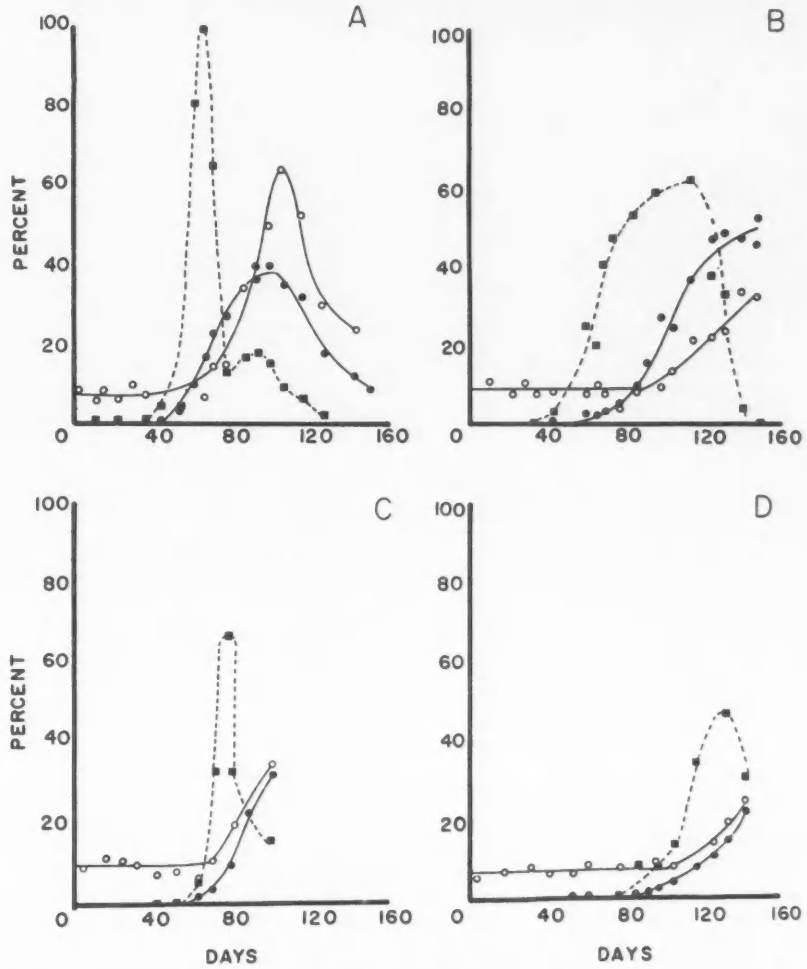

FIG. 4. Time course of experimental VEN infection in adult Atlantic cod (four individuals from two experiments). ■, VEN-infected immature erythrocytes; ●, total erythrocytes infected; ○, uninfected, immature erythrocytes.

mental transmission varied among the different experiments; however, the number of infected fish showing evidence of VEN by microscopic examination was 31/63 (33%). Fish that were mock infected by inoculating with erythrocytes from VEN-negative donors did not develop VEN.

At the microscopic level, the appearance of ENV-infected cells in experimentally infected cod was identical to that observed in erythrocytes of naturally infected fish. Infected erythrocytes showed evidence of severe nuclear degeneration including pyknosis, karyorrhexis, and karyolysis and the characteristic circular, eosinophilic inclusion body, with satellite punctiform bodies that are probably individual virions (Fig. 2). Examination of infected erythrocytes by electron microscopy revealed typical ENV: 300–350 nm diameter hexagonal and pentagonal particles with a complex capsid and core structure (Fig. 3). By these criteria, the erythrocytes appeared to have been infected with ENV.

A more extensive experiment was carried out in order to identify the cell type(s) initially infected and follow the progression of ENV pathogenesis. Cod were experimentally infected with ENV by both intravenous and intraperitoneal injection or mock infected and each was sampled at weekly intervals to determine their VEN status. The results of this experiment are shown in Table 2. Three of four fish inoculated with washed, infected erythrocytes became VEN positive about 1 mo postinoculation. All three had been exposed by intraperitoneal injection, while the fish that had been exposed by intravenous injection into a caudal vessel remained VEN negative. A single fish inoculated intravascularly with washed erythrocytes from a VEN-negative donor became infected; however, the rapid onset of VEN (7 d) indicated that this individual probably was in the incubation phase of the disease at the time of exposure.

As depicted in Fig. 4, the overall pattern of pathogenesis of the experimentally induced infectious process followed that of the natural infection; there was an initial heavy infection of immature erythrocytes followed by evidence of infection of mature erythrocytes, and finally, erythroblastosis consisting

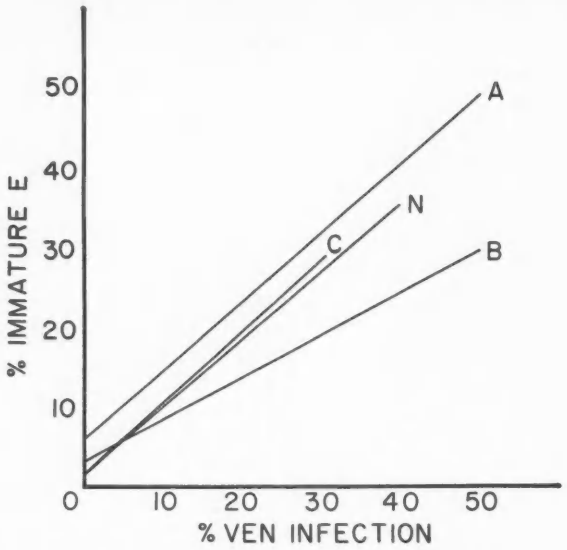

FIG. 5. Linear regression analysis of % VEN infection rate versus % immature erythrocytes. N = naturally infected fish; A, B, and C = experimentally infected fish. Equations for lines: N: $r = 0.812$, $Y = 0.76x + 3.26$; A: $r = 0.902$, $Y = 0.73x + 6.1$; B: $r = 0.866$, $Y = 0.52x + 4.1$; C: $r = 0.95$, $Y = 0.715x + 3.40$.

of VEN-negative immature erythrocytes. In both experimentally infected and naturally infected fish, linear regression analysis indicated a significant ($P < 0.05$) positive correlation between the level of immature erythrocytes and percent of infected erythrocytes (Fig. 5). Some variability in the patterns of infection of individual fish was observed. For example, although the time interval during which immature erythrocytes were seen to be infected was generally very short, one fish showed evidence of infected immature erythrocytes over virtually the entire sampling period (Fig. 4B). Also, whereas most fish progressed rapidly from a barely detectable erythrocyte infection rate (<0.001%) to the maximum rate, one individual (Fig. 4D) remained at a low level of infection for more than 3 mo. In contrast with the high levels of immature erythrocytes in infected fish, the proportion of these cells in the peripheral blood of over 100 VEN-negative fish examined microscopically was low (usually in the range of 5–10% and never exceeding 15%).

Discussion

This study has demonstrated experimental transmission of VEN in Atlantic cod by inoculation of either washed infected erythrocytes or a cell-free lysate of infected cells. Successful transfer of disease occurred in four of five experiments. There was no obvious relationship between the intensity of infection in the donors and the success rate of transmission, although in the experiment where infection was not successful the donor fish had the lowest infection rate (2%). The overall rate of successful transmission of VEN was 33%, 60–90% lower than that found in intraspecific transfer of VEN in *Oncorhyncus* (MacMillan and Mulcahy 1979). This difference may be accounted for by the differences in the species used, and by the differences in age of the recipient fish in the two studies. MacMillan and Mulcahy (1979) found a strong relationship between the size of the recipient fish and both the time to first evidence of infection (2 d for 0.3-g fish, 20 d for 25-g fish) and for the intensity of infection generated (60% for 0.3-g, 40% for 25-g chum salmon (*O. keta*)). Our experiments were limited by necessity to adult fish (30–50 cm total length) which may have reduced the success rate and intensities of infection. In addition, the viruses present in the erythrocytes of the two species are morphologically quite distinct (350 nm in cod, 145 nm in *Oncorhyncus* and *Clupea*) and may have variable effects on the respective hosts.

The relatively low success rate of experimental transfer of infection may have been due to host defense mechanisms. Since the fish used in these experiments were feral, no disease histories were available for the individuals used and some may, in fact, have had VEN and recovered. Thus, some animals may have been immune and therefore refractory to reinfection. In this respect, it is interesting to note that erythrocytes from one donor fish which had recovered from VEN failed to transmit the disease to recipients (Table 2).

The large size of cod ENV, 350 nm (Appy et al. 1976; Reno and Nicholson 1980; Walker and Sherburne 1977), precluded filtration through bacteria-retaining filters as is usually done when attempting to determine viral etiology. However, the unique morphology and location of the agent which was found consistently with the presence of erythrocytic lesions indicate that ENV was transmitted in these experiments.

The time course of VEN infection, both natural and experimental, was protracted, with an extended incubation period (approximately 1 mo) as well as a lengthy disease phase of about 3 mo duration. In all instances in which the infection was followed over a long period of time, complete resolution occurred with no indication of infected erythrocytes remaining. The disease, while chronic and nonlethal to fish held in captivity, has deleterious physiological consequences including anemia (Reno et al. 1985), which may seriously affect survival in the wild.

The most important finding was that the first cells to exhibit infection were immature erythrocytes, generally those in the final stages of maturation. This is the first evidence that erythrocyte precursors rather than the fully mature erythrocytes themselves are the sites for initiation of ENV infection. The course of infection appeared to begin in a population of immature erythrocytes and became evident as intracytoplasmic inclusions and nuclear degradation approximately 1 mo after exposure. In some instances, virtually all immature erythrocytes in the peripheral circulation were infected. There are no data in the literature that detail the rate of erythropoiesis in Atlantic cod. Consequently, it is difficult to assess the maturation rate of erythrocytes in this species and, therefore, to more percisely determine at what stage of erythrocyte maturation ENV invades the cell. It is likely, however, that the infection is established at least 1 mo prior to visible lesions in the erythrocyte.

The erythroblastosis which occurred during the latter stages of the infection was probably elicited by the decline in the functional capacity of ENV-infected erythrocytes. In order to maintain adequate levels of oxygen in tissues, this adjustment was probably necessary. Other studies in our laboratory (Reno et al. 1985) have indicated that VEN causes a moderate normocytic anemia in Atlantic cod. This is in contrast with the severe anemia caused by VEN in Pacific salmon (MacMillan and Mulcahy 1979). We have found no evidence of VEN in the immature erythrocytes generated late in infection. Whether the lack of infection at this stage was due to specific immu-

nological defenses or to insufficient duration of observation remains conjectural. However, while maintaining cod obtained during the declining VEN phase (often for more than 3 mo), recrudescence of the disease has not occurred (P. W. Reno, unpubl. data).

Further work should be carried out on this disease to determine its impact on susceptible populations, its mode of transmission in the sea, and its potential for interspecies transfer as well as in the basic biochemistry and immunology of the viral agent. The chronic nature of this disease may lead to subtle detrimental effects on cod populations in comparison with more lethal diseases but these effects may, nonetheless, have a severe impact on cod populations.

Acknowledgements

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Diel Vertical Movements and Feeding Activity of Zooplankton in St. Georges Bay, N.S., Using Net Tows and a Newly Developed Passive Trap

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Newly developed plankton traps, designed to passively collect vertically mobile plankters, sampled ascending plankton but failed to collect many species during descent. This discrepancy may be behavioral with passively sinking organisms reacting to the trap surface with an upward avoidance reaction. Simultaneous use of conventional net tows and semiautomated traps allowed us to interpret the vertical movements of plankton more fully than by either method alone. Asynchronous vertical movement of the *Pseudocalanus* population is suspected because the percentage of trapped animals with food in their guts was usually higher in the downward moving fraction of the population. Migratory behaviors ranged from dusk and dawn ascent with midnight sinking to reverse migrations where the night level inhabited is deeper than the day depth. Noctural dispersal of herbivore and omnivore populations over depth probably reflects predator avoidance by presenting less dense aggregations to vertically mobile predators. Sightless predators reside in deeper waters than their prey during daylight presumably because they are larger and more vulnerable themselves to visual predation. Visual predators descend to greater depths than their prey at night. All the migration patterns observed can be explained in evolutionary terms simply by competition for food and avoidance of predators.

Les pièges à plancton récemment mis au point, conçus pour recueillir passivement les organismes planctoniques à mobilité verticale, ont permis de prélever des échantillons de plancton ascendant, mais n'ont pas permis de recueillir nombres d'espèces durant la descente. Cette divergence pourrait être de nature comportementale, les organismes à descente passive réagissant à la surface du piège par un mouvement d'évitement vers le haut. L'utilisation simultanée de filets conventionnels et de pièges semi-automatiques nous a permis d'interpréter les déplacements verticaux du plancton de façon plus complète que par l'une ou l'autre méthode seule. On présume que le déplacement vertical de la population de *Pseudocalanus* est asynchrone, car le pourcentage des animaux pris avec des aliments dans l'intestin était généralement plus élevé dans la fraction descendante de la population. Les comportements migratoires variaient de la montée au crépuscule et à l'aube, avec descente au milieu de la nuit, aux migrations inverses lorsque le niveau d'habitat de nuit est plus profond que celui du jour. La dispersion nocturne des populations herbivores et omnivores en fonction de la profondeur reflète probablement l'évitement de la prédation en présentant des agrégats moins denses aux prédateurs à mobilité verticale. Les prédateurs aveugles se trouvent en eau plus profonde que leurs proies durant le jour probablement parce qu'ils sont plus gros et donc plus vulnérables eux-mêmes à la prédation à vue. Les prédateurs à vue descendent à de plus grandes profondeurs que leurs proies la nuit. Tous les modes de migration observés peuvent s'expliquer en termes d'évolution par la simple concurrence alimentaire et l'évitement des prédateurs.

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Written accounts of the surface swarming of aquatic organisms date back to at least the beginning of the nineteenth century when Cuvier observed that daphnids occurred at the surface during the morning and evening on bright days (see Cushing 1951). Since Michael's (1911) work on the vertical migration of chaetognaths most investigators have used sequential water samples or horizontal net tows at various depths to study the diel movement of plankters. However, indirect analyses of this type, using changes in population depth modes to denote movements, depends on synchronous movement by most members of a population; otherwise it could lead to erroneous conclusions about the strength and even existence of vertical migration (Pearre 1979). An asynchronous population which undergoes an extensive vertical migration but spends little time at either or both its depth extremes or in transit would yield seemingly static depth distributions from serial samplings. Obviously, this is because an individual's probability of capture is greatest at the depth or depths at which it spends most of its time. The plankton literature abounds with examples of "static" populations with bimodal, skewed, or uniform depth distributions which may have been incorrectly used to infer either weak

or nonmigratory species or development stages (e.g. Russell 1925, 1926; Clarke 1934; Hure and Scotto di Carlo 1974; Lee and Williamson 1975; Sekiguchi 1975; Youngbluth 1976; Hopkins and Gulliksen 1978; Southward and Barrett 1983; Williams and Conway 1984).

A timely paper by Bovbjerg et al. (1976) proposed using a passive collector, vertically orientated net traps, to allow a direct measure of vertical migration expressed as the number of individuals moving up or down through a known area over a predetermined time period. Some inference is still needed because movement may be local and random. However, the extent of any migration might be clarified by the deployment of several traps over a broad depth range. This technique has been long used to quantify aquatic insect emergence (Needham 1908) but, as far as we know, it has not been used to study marine plankton migrations. Recently, marine benthic investigators (Thomas and Jelley 1972; Alldredge and King 1977; Youngbluth 1982) have adopted and modified the freshwater emergence trap concept (Mundie 1971; Morgan 1971) to study diel migrations of epibenthic organisms into the water column. We developed a clear, semiautomated, opening–closing trap for collecting both ascending and descending open-water plankton populations (Vass et al. 1981). In the present report we describe the first results of a 24-h vertical migration and feeding study on the neritic zooplankton population in St. Georges Bay, N.S., using both the horizontal net tow and ascendant–descendant trap techniques.

Methods

St. Georges Bay is a shallow marine embayment open on the north to Northumberland Strait and the southern Gulf of St. Lawrence. A 24-h field investigation of the diel vertical movement of plankton was undertaken near the mouth of the Bay (45°50′N, 61°48′W) in ~33 m of water between 08:00 August 19 and 10:00 ADT August 20, 1980. The sky throughout the study was clear with a high altitude haze present. The wind was less than 10 knots from the northeast on the first day, dying out in the late afternoon, calm throughout the night, and then increasing to ~5 knots from the south by 08:00 the next morning. A half moon was present until shortly after midnight. Alternate low and high water levels occurred at 08:54 (0.8 m, above chart datum), 15:29 (1.1 m), and 20:14 (1.1 m) August 19 and 02:29 (1.3 m) and 10:04 (0.8 m) August 20 with the extreme range being 0.5 m (Canadian Hydrographic Service 1980).

Temperature, salinity, plant pigments, and nutrients data were collected at 12:25 August 19 and 00:25 August 20 at 5-m depth intervals using Nansen bottles with reversing thermometers. Salinity samples were later analyzed using an Auto-lab® salinometer. Temperature and conductivity data were also available from an Aanderaa® thermistor chain moored in the Bay throughout the summer. Chlorophyll *a* and pheopigments were measured on a Turner fluorometer following the techniques of Holm-Hansen et al. (1965). Nitrate, silicate, and phosphate samples were analyzed with standard colorimetric methods (Strickland and Parsons 1972). Light penetration was measured throughout the water column at 14:00 with a LI-COR® model LI-185 B quantum meter and LI-192 SB underwater sensor. The ^{14}C fixation method outlined by Strickland and Parsons (1972) was used to measure in situ phytoplankton primary production at 5-m depth intervals (see Marine Ecology Laboratory 1980). Incubations started at 09:30 and ended 13:30 August 19. Incident radiation was recorded on deck throughout this study with a LI-COR® LI-550 integrator, set for 10-min intervals. Daily carbon fixation at each depth was extrapolated from the fraction of radiation received during the incubation period.

Transparent traps were designed and built to collect vertically mobile plankters in the field. Detailed material, design, and construction specifications have been documented (Vass et al. 1981). Each trap unit consists of a down-facing and up-facing pyramid-shaped funnel (0.16-m^2 openings) to concentrate and direct ascending and descending plankters into separate 2.6-L collection chambers. These chambers are opened and then closed at depth by a double-messenger tripping mechanism, through the sliding action of a spring-loaded gate valve. In this study, four trap units were moored at 8, 14, 21, and 28 m depth (Fig. 1). The mooring was kept taut between a train wheel and subsurface float. During deployment the collection chambers are filled with GF/C-filtered seawater and lowered in the closed position to avoid surface contamination. Self-opening flaps on the walls of the collecting funnels ensure flushing during deployment. The battery operated messenger-release timer is activated on deck before deployment. This timer is programmed to release a narrow messenger 30 min after activation and a broad messenger 4.5 h later. The first messenger strikes the trip bar closest to the mooring wire which allows the gate valve to slide to the open position and release a similar messenger beneath the first trap. This process continues until all four traps are opened. The traps are allowed to collect mobile plankters for 4 h after which the subsurface timer releases a broad messenger. This strikes a second trip bar further from the wire which releases the gate valve to slide to a closed position. Similarly, this initiates the closing process for the other three traps. The entire assembly can be retrieved on deck, trapped plankton preserved in 5% buffered formalin solution, and redeployed within an hour. In all, four deployments were made during this study. The collection times of 11:00–15:00, 17:00–21:00, 23:00–03:00, and 05:00–09:00 ADT were chosen to coincide with day, dusk, night, and dawn light conditions. All trapped plankton were identified to species and developmental stage although only the more abundant forms can be used here to decipher vertical migrations.

The value of having clear plankton traps was tested on two separate days the following year, August 18 and 20, by covering two of our four traps with black plastic. All four traps were deployed at 14 m depth on separate moorings within a 5000-m^2 area in St. Georges Bay. The clear and dark traps were opened simultaneously (±3 min) and allowed to collect plankton for 4 h between 11:00 and 16:00.

Eight series of Clarke-Bumpus net (12-cm diameter, 333-μm mesh) tows of 4–5 min duration were taken in 1980 to overlap with the opening and closing times of the plankton traps. A series comprised seven separate horizontal tows at 5-m depth intervals; each vertical series required about 1 h. The order of towing was always from shallowest (1 m) to deepest (30 m) depth. The tow depths were estimated from wire angle and length of wire out and verified at the end of each tow from a Benthos time-depth recorder (No. 1170E) trace. Even the deepest tows were within 1 m of the desired depth. Each net was soaped and/or spray cleaned before use, thus eliminating cumulative clogging problems. Average filtration during a tow varied little over our sampling scheme, 5.37 ± 0.76 m^3 ($\bar{x}$ ± SD) seawater. Time did not allow duplicate tows but it is

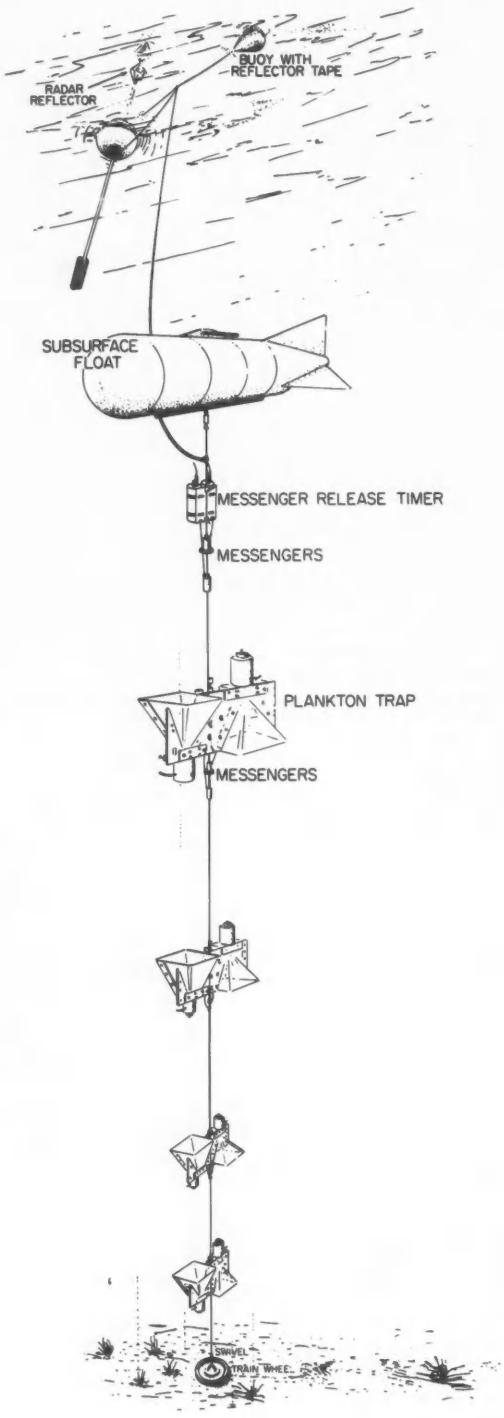

FIG. 1. Deployment of mooring with four traps (see text).

hoped that the tow durations were sufficiently long to even out some small scale patchiness. The most abundant forms were subsampled with a Motoda splitter because of large sample size. We calculated fiducial limits of subsampling (Horwood and Driver 1976) to arrive at the least number of sample splits needed to distinguish significant changes in a species abundance over depth. Only common species are reported here. In interpreting population depth shifts, we found that some distributions were asymmetrical. We therefore decided to use population depth medians and quartiles, following the example of Pennak (1943) and Bosch and Taylor (1973). Excessive patchiness caused us to follow earlier workers by reporting depth–frequency distributions as percentages. The dominant species caught in both trap and net tows were visually assessed as to the presence or absence of food in their digestive tracts by clearing the organisms in glycerin. The guts of fish larvae were dissected and examined.

Physical and Biological Conditions during the Field Experiment

Past studies have shown that the thermally homogeneous surface layer gradually deepens from 10 m in June to >30 m by late October (Hargrave et al. 1985). During our 24-h field experiment the upper mixed layer was nearly isothermal (18.7–17.3°C) and isohaline (29.4–29.5‰) down to ~25 m depth (Fig. 2). The bottom layer was cooler (17.3–13.6°C) and slightly more saline (~29.6‰) with a vertical extent of only 8 m at our sampling location. Past studies indicate that this stratified condition is persistent and widespread in the Bay, altered only by the available depth of water and season. Dissolved nitrate (0.48 ± 0.07 $\mu Mol \cdot L^{-1}$) and phosphate (0.54 ± 0.03 $\mu Mol \cdot L^{-1}$) concentrations were low throughout the upper mixed layer, both day and night (Fig. 2). Silicate concentrations (4.8 ± 0.3 $\mu Mol \cdot L^{-1}$) were uniformly high in the upper layer as expected from our previous study (Hargrave et al. 1985). A strong gradient of all three nutrient concentrations existed below the thermocline, increasing towards the sediment surface (Fig. 2). The apparent day–night differences in temperature, salinity, and nutrient concentrations at 30 m depth (Fig. 2) are an artifact caused by the tidal change in the water depth.

The water column was very turbid due to previous strong northeast winds which resuspended sediments from the shallow margins of the Bay. Only 57% of the surface radiation reached 1 m depth, which approaches the most turbid coastal waters observed (Sverdrup et al. 1942). The mixed layer was deepened 8 m by the storm and exceeded the euphotic zone by about 5 m at the start of the field experiment. In 1977, carbon assimilation by phytoplankton increased gradually throughout the summer with maximum rates in August through September (Hargrave et al. 1985). The level of primary production in the present study is similar to the maximum rates observed in 1977, 455 mg $C \cdot m^{-2} \cdot d^{-1}$, and was highest between 5 and 15 m depth. There was no accumulation of phytoplankton stocks, as measured by plant pigments, over the day–night period, suggesting a rapid consumption by herbivores. Previous studies of the biological dynamics in St. Georges Bay indicate a rapid, tight coupling between nutrient regeneration, phytoplankton production, and consumption by herbivores and in turn by carnivores (Hargrave et al. 1985).

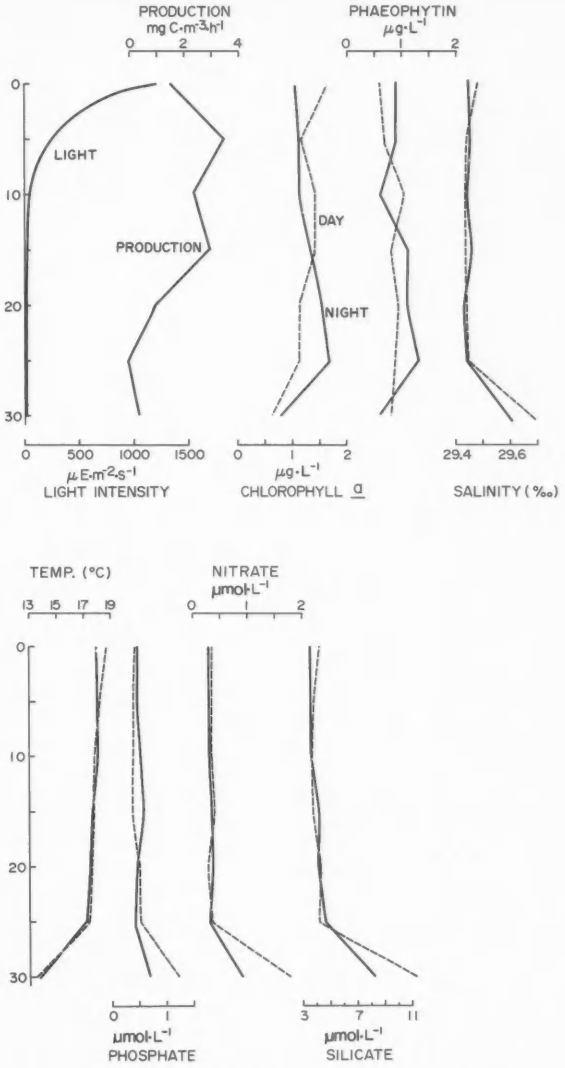

FIG. 2. Vertical depth profiles of light, primary production, salinity, and temperature and chlorophyll *a*, phaeophytin, phosphate, nitrate, and silicate concentrations during a day (broken line, August 19) and night (solid line, August 20) sampling.

Zooplankton Distributions, Movements, and Feeding Periodicity

The following is a descriptive account of the observed vertical movements and feeding periodicity of each of the common species in St. Georges Bay, discussed in context with previous studies. The order in which a species is presented depends on its migration pattern, starting with the complex dusk and predawn rises between midnight sinking (e.g. *Pseudocalanus*), through a series of intermediate patterns, to the so-called reverse migration where the night level inhabited is deeper than the day depth (e.g. *Palaemonetes* and *Cancer*).

Pseudocalanus minutus

The patchy distribution of this species resulted in an order of magnitude change in abundance after the third series of horizontal tows (Fig. 3). Although this change coincided with the dusk ascent, we can find no evidence of deep transit from either the depth–frequency diagrams or trap collections which one would expect if we had missed sampling a significant proportion of the population close to the bottom during daylight.

Adult females and stage V copepodids were concentrated in the upper 15 m but the range of this species extended to the bottom (Fig. 3). Adult males were located deeper in the water column and their population was more dispersed (Fig. 3). All three stages had a tendency to rise towards the surface at dusk, submerge by midnight, and rise again before dawn. The final descent to daytime depths started at least 2 h before dawn. This migration behavior, known as "midnight sinking," has been reported for many species of marine plankton and it is usually ascribed to a lack of orientation by the plankters during darkness (see Cushing 1951) but also due to satiation (Pearre 1973). In our case a bright half moon was present until after the midnight sampling; thus there would be a selective advantage to early descent, once satiated, to avoid visual predators.

The trap results, in general, support our interpretations of the vertical migration of *Pseudocalanus* from population depth analysis (Fig. 3). The daytime upward component at 8 m depth appears to be overly high given that the traps were located outside a patch and there was no apparent change in the population's depth distribution over the same time. The dusk rise is nicely recorded by the traps. However, the predawn rise is inadequately represented in the trap collections, especially considering the large numbers of *Pseudocalanus* present in the water column. The postdawn descent of the population is well represented by the trapping results.

Our observations on the gut contents of trap and net collected zooplankton species demonstrate that many individuals had defecated within the traps because fewer of the trap-collected organisms had material in their digestive tracts (e.g. Table 1). Further discussion of feeding periodicity, therefore, is based on gut observations made on net-collected individuals (Table 2). In our 24-h study, female *Pseudocalanus* fed mainly at night although greater than 20% of the population was feeding at any time of the day (Table 2). Stage V *Pseudocalanus* fed equally well during dusk and nighttime with 14 and 18% of the population feeding during daytime and dawn periods, respectively. *Pseudocalanus* males had empty guts throughout this study, which suggests a solely reproductive function at this time of the year.

Previous field studies have all shown that *Pseudocalanus* feeds mainly during the dusk and dawn periods (Wimpenny 1938; Mackas and Bohrer 1976; Nicolajsen et al. 1983). Bohrer (1980) has described dusk and dawn surface migrations by captive female *Pseudocalanus* in a 10-m tower tank when food was either absent or at low concentrations (1–2 mg Chl $a \cdot m^{-3}$). This population was dispersed deeper in the water column during the nighttime at these low food concentrations. At chlorophyll *a* concentrations > 2 mg $\cdot m^{-3}$, Bohrer found the night rise was unimodal, occurring in the first half of the night and followed by a gradual sinking of the population throughout the night. Chlorophyll *a* concentrations in the present study were low (1.0–1.5 mg $\cdot m^{-3}$) and uniform throughout the upper 25 m (Fig. 2). Thus, the observed migration pattern of *Pseudo-*

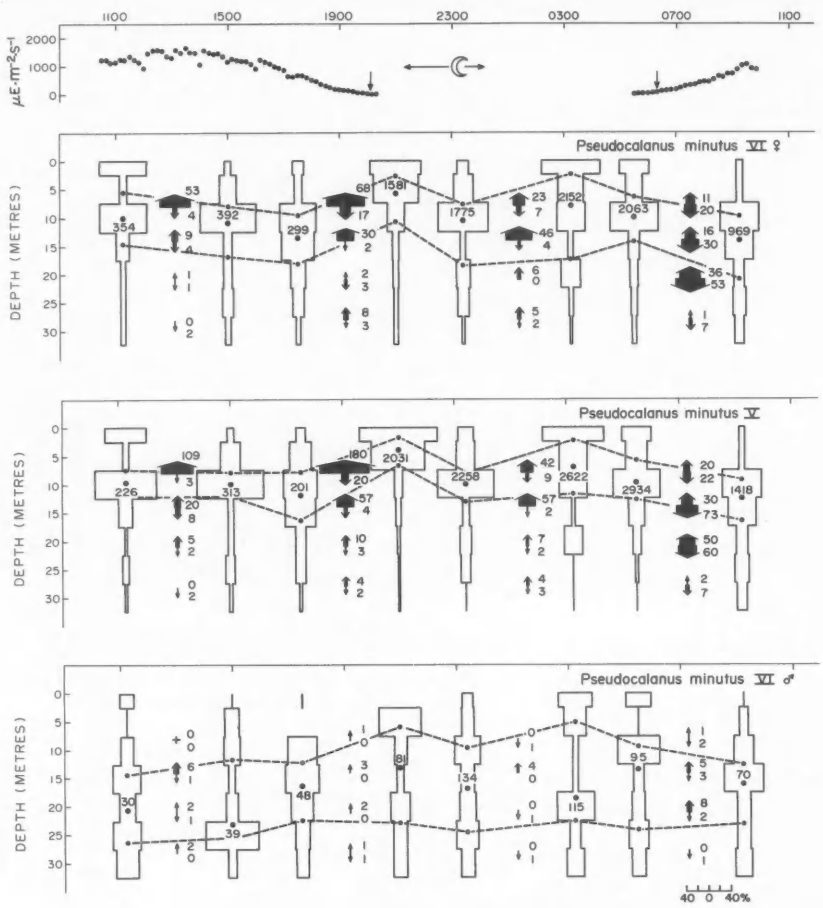

FIG. 3. Depth–frequency diagrams and trap results (dark arrows) of *Pseudocalanus minutus* females, males, and stage V copepodids for eight and four sampling periods, respectively, between 10:30 August 19 and 09:30 August 20, 1980. Incident radiation recorded on deck is indicated in the upper panel; arrows indicate sunset and sunrise. The actual number of individuals moving up or down into the traps at 8, 14, 21, and 28 m depth is indicated next to each arrow. Trap deployment and retrieval time was set to coincide with the middle of adjacent horizontal, net-tow series. The 25 and 75% population quartiles are connected by broken lines and the population median is indicated by a black dot. The number located inside each depth–frequency diagram (scale lower right) is the estimated number of individuals $\cdot m^{-3}$ in the water column during this series of horizontal net tows.

TABLE 1. Comparison of diel feeding patterns (% with food in gut) obtained from observations of plankton net- versus trap-collected zooplankters (N = sample size).

		Day		Dusk		Night		Dawn	
	Gear	%	*N*	%	*N*	%	*N*	%	*N*
Temora	Net	97	300	98	350	98	319	95	299
longicornis, ♀	Trap	57	684	75	253	69	235	71	217
Pseudocalanus	Net	31	199	26	286	56	343	20	350
minutus, ♀	Trap	1	74	5	117	30	93	42	174
Centropages	Net	77	117	95	85	90	83	78	89
hamatus, ♀	Trap	8	119	18	44	15	66	40	115
Podon	Net	27	89	68	73	52	145	33	114
intermedius	Trap	11	101	55	30	20	41	3	63

TABLE 2. Diel feeding patterns (% with food in gut) of net-collected specimens (N = sample size).

	Day (10:45–15:30)		Dusk (17:00–21:30)		Night (23:00–03:30)		Dawn (05:00–09:30)	
	%	N	%	N	%	N	%	N
Evadne	5	100	31	83	36	159	38	113
Bivalve larvae	34	243	22	242	31	202	39	208
Podon	27	89	68	73	52	145	33	114
Pseudocalanus[a]								
♀	31	199	26	286	56	343	20	350
V	14	102	43	211	49	315	18	325
Temora								
♀	97	300	98	350	98	319	95	299
♂	93	277	98	336	97	317	94	245
Centropages								
♀	77	117	95	85	90	83	78	89
♂	55	86	70	101	65	109	68	93
Tortanus								
♀	27	11	30	30	28	29	28	18
♂	0	36	7	61	11	28	9	33
Calanus IV, V	25	16	56	16	30	47	19	58
Cancer zoea	20	5	60	5	54	13	45	11
Sagitta	12	59	25	52	37	78	18	98
Palaemonetes larvae	67	21	73	30	60	30	48	21
Tautogolabrus larvae	40	10	15	13	0	9	25	4

[a]*Pseudocalanus* males did not feed, N = 125.

calanus in St. Georges Bay is consistent with the findings of Bohrer at low chlorophyll levels. In the Bay, *Pseudocalanus* occupies different depths of the euphotic zone at different times of the day and appears to utilize phytoplankton from the entire productive zone even though feeding is at least partly restricted in time.

Temora longicornis

The abundance of *Temora* was less variable (~5 times) than *Pseudocalanus*, with no broad patch moving through the sampling area. Adult populations were concentrated near 10 m during daylight; however, the males were dispersed deeper in the water column (Fig. 4). At dusk, adult *Temora* migrated towards the surface, their median population depths reaching ~5 m by 21:00. Small female *Temora* (<0.78 mm cephalothorax length) dispersed at dusk, being equally abundant from the surface down to ~15 m depth. By midnight the populations of both sexes were centered below daylight levels and dispersed over a broader depth range. Between midnight and 03:00, *Temora* rose again, as did *Pseudocalanus*, except that the median depth reached by each developmental stage did not exceed its daytime depth. *Temora* remained dispersed until 09:00 the next morning. *Temora longicornis* is well known as a coastal species which migrates towards the surface at night within the upper water mass (Savage 1926; Pearre 1970; Atherton 1979). Bohrer (1980) has experimentally produced dusk and dawn rises with adult *Temora* contained in a 10-m tower tank.

Few *Temora* were caught swimming down into the traps compared with the large numbers swimming towards the surface (Fig. 4). Earlier experimental work has shown that *Temora* can swim directly up and down, with little lateral movement, and was observed to undergo sustained upward but not downward migration (Hardy and Bainbridge 1954). Anderson

TABLE 3. Upward movement of zooplankters (no. $\cdot 4h^{-1} \cdot 0.16\ m^{-2}$, ± SD, N = 4) during daylight, between 10:00 and 16:00, into clear and darkened traps at 14 m depth, August 18 and 20, 1981, St. Georges Bay (ns = not significant).

	Clear traps	Darkened traps	ANOVA
Pseudocalanus minutus			
♀	19.0±14.5	13.8±13.6	ns
♂	5.5±1.9	6.3±3.0	ns
V	25.5±14.8	18.3±14.9	ns
Temora longicornis			
♀	12.8±3.9	33.5±11.5	0.02–0.01
♂	17.3±4.4	43.8±20.6	0.05–0.02
V	1.5±1.3	3.8±5.0	ns
Podon intermedius	11.5±4.4	12.8±9.5	ns
Evadne nordmanni	3.8±3.0	5.8±6.3	ns
Tortanus discaudatus			
♀	0.8±1.0	2.5±2.4	ns
♂	0.5±1.0	2.3±3.3	ns
Centropages hamatus			
♀	2.8±2.4	2.0±1.4	ns
♂	1.5±1.3	3.0±1.4	ns
Calanus finmarchicus			
V	1.3±1.3	0.5±0.6	ns
Oithona similis			
♀	22.3±11.7	24.3±11.5	ns
♂	13.0±3.9	9.8±3.2	ns
Juveniles	98.3±32.8	96.5±20.7	ns

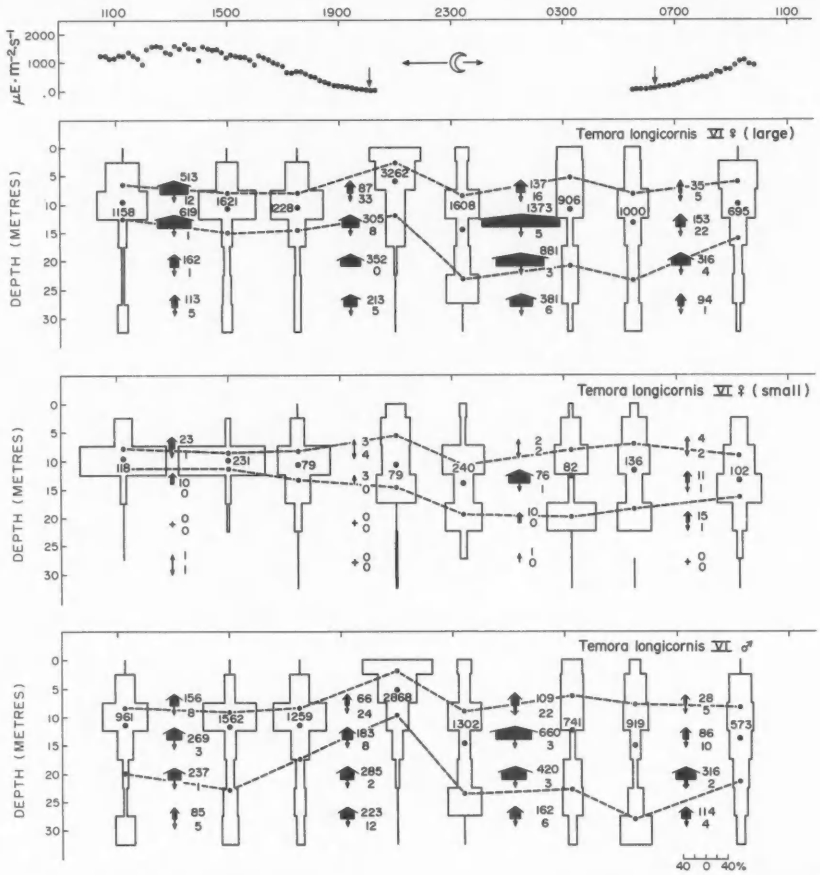

FIG. 4. Depth–frequency diagrams and trap results of *Temora longicornis* large and small females and males during the 24-h study (see Fig. 3 for explanation).

(1974), however, found that *Temora* swam slightly more frequently in the horizontal than vertical plane in less confined experimental conditions. It appears likely that *Temora* descends passively and its response to contacting a solid surface is an upward swimming reaction. The most intense upward movement into the traps occurred at night when the population was deep but dispersed. This vertical movement may be local or may extend over the entire range of depths occupied at night. *Temora* is also very active during daylight, according to the trap collections, but their vertical movement is restricted because 50% of the population was concentrated at 10 ± 2.5 m depth. Adult *Temora* were the only zooplankters which showed a greater upward movement into darkened traps than into clear traps (Table 3). It is possible that *Temora* was responding to the measured 24% reduction in light beneath the clear traps (Vass et al. 1981) during daylight. The dusk ascent observed from the depth frequency diagrams is not well represented by the trap collections. The traps are useful for studying only the upward movement of *Temora*.

Adult *Temora* females and males fed continuously throughout this study with over 90% of the individuals having fed at any particular period of the day (Table 2). Wimpenny (1938) also found that this species fed continuously throughout a 24-h period in June from simple shipboard experiments done in the North Sea. However, Mackas and Bohrer (1976), using gut fluorescence techniques, found a slight indication of increased feeding activity in *T. longicornis* around sunset in Bedford Basin, N.S., in March. Head et al. (1984), using the same techniques, similarly observed maximum feeding between midnight and 06:00 in July at all depths in St. Georges Bay. In tower tank studies lasting 6 d, Bohrer (1980) demonstrated that *Temora* rose continuously from early morning to dusk, and then descended slightly throughout the night with some indication of a small dawn rise before descending to the deepest morning depths. The addition of various concentrations of phytoplankton at the surface did not appear to consistently modify the vertical extent of this migration. However, a subsurface (~3 m depth) addition of phytoplankton did abbreviate the "dusk" ascent of the population. This effect was not observed in St. Georges Bay because the low phytoplankton biomass was uniformly distributed throughout the water column. *Temora*, however, was located within the depths of maximum phytoplankton production during daylight and migrated throughout the production zone at night, thus utilizing the entire water column for food.

Bivalve Larvae

No attempt was made to identify bivalve larvae because of

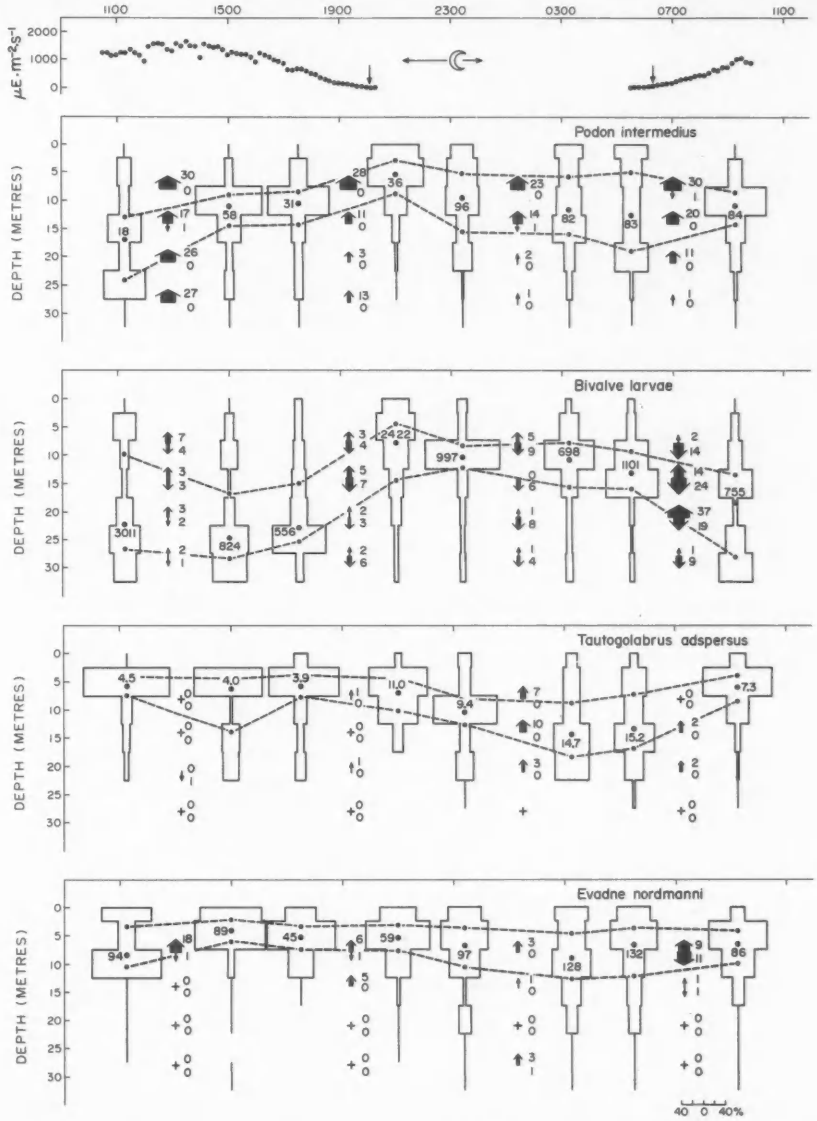

FIG. 5. Depth–frequency diagrams and trap results of *Podon intermedius*, bivalve larvae, *Tautogolabrus adspersus*, and *Evadne nordmanni* during the 24-h study (see Fig. 3 for explanation).

the difficulty involved; however, *Mytilus* was a dominant species in our collections. The average bivalve larval abundance in the water column varied by as much as 5 times over the 24-h period but bore little resemblance to the patchiness of other species in these collections. The median population depth of all bivalve larvae was below 20 m depth during the day but rose sharply at dusk to reach 7.5 m by 20:00, and then remained at ~10 m depth throughout the night (Fig. 5). At dawn the bivalve population descended but had not reached daytime levels by 09:00. Plankton trap collections show that considerable vertical movement occurred throughout the 24-h period, particularly during 05:00–09:00, but fail to corroborate the strong dusk rise and dawn descent indicated by population depth–frequency analysis. Bivalve larvae fed equally (30–40% of the population), during day, dawn, and night periods, but only 22% fed during the dusk ascent.

Bivalves are believed to have evolved a vertical distribution cycle based on the tides which enables the species to be retained within an estuary where surface waters flow seaward above a deeper countercurrent (see Bayne 1979). The diel pattern of vertical migration found here for bivalve larvae has not been reported before in coastal waters.

Podon intermedius

Podon was twice as abundant in the tows taken between midnight and dawn compared with previous sampling during day and dusk (Fig. 5). Apart from the 11:00–12:00 sampling period, *Podon* had a compact daytime distribution centered at

the 10 m depth. The population migrated into the upper 5 m at dusk, followed by a rather even midnight dispersion over the upper 15 m. This vertical distribution lasted until dawn when the population rose and concentrated again around the 10 m depth level. Considerable upward movement of *Podon* into our traps occurred throughout the 24-h period but this may have been short in vertical extent. *Podon* fed mainly over the dusk and nighttime periods with only 30% of the population with food present in the guts during dawn and day periods (Table 2). We have been unable to locate any previous studies of the diel vertical migration of this species but its behavior is similar to its congeners (Hansen 1951; Atherton 1979).

Evadne nordmanni

Evadne abundance varied by a factor of three but there was no discernible trend over the eight sampling periods (Fig. 5). The *Evadne* population accumulated near the 5 m depth level during daylight, dispersed after dusk to occupy the upper 20 m, and then returned to daylight depths by 09:00. The trap results indicate that a substantial vertical exchange occurred, both up and down, over the dawn period. Reports in the literature are about equally divided with *Evadne* either migrating towards or away from the surface at night (e.g. Lee 1974; Burris 1980). Thirty to 40% of the population had food in their guts between dusk and dawn with only 5% having fed during the day (Table 2). This is contrary to Bainbridge's (1958) results from Loch Fyne where 30−50% of the *E. nordmanni* were feeding during daylight and 0−3% after dark.

Tautogolabrus adspersus

The abundance of larval cunners (3−11 mm total length) was 3 to 4 times greater during the dusk to dawn period which corresponds to the plankton patch we believe passed through our sampling area. During daylight, larval cunners accumulated around the 5 m depth level although scattered individuals were found down to 20 m (Fig. 5). At dusk the population submerged until the center of abundance reached 15 m (03:00−06:00) and then returned to daylight depths by 09:00. Larval cunners were caught swimming up when stratified net tows indicated that the population was dispersing down (23:00−03:00) which emphasizes the value of using both techniques in migration studies. Most teleosts studied to date have a "normal" migration into shallower water at night (Smith et al. 1978; Kendal and Naplin 1981; Southward and Barrett 1983) although Russell (1926) found two species with a reverse migration similar to our findings on the cunner. Feeding occurred only during the daytime when 40% of the individuals examined had fed (Table 2). This is consistent with the idea that fish larvae use vision to capture prey (Blaxter and Holliday 1963).

Tortanus discaudatus

Twice as many *Tortanus* occurred in the water column between midnight and 03:00 compared with the abundance at other times (Fig. 6). Adult *Tortanus* were distributed evenly throughout the water column between 5 m and the bottom during daylight, with males more abundant in the deepest tow. At dusk the entire population moved up, maintaining an even distribution throughout the upper 20 m of the water column. There was little indication of midnight sinking in this species. *Tortanus* returned to daytime depths between 06:00 and 09:00 the next morning. *Tortanus* was too rare to be sampled adequately by the plankton traps but they show some vertical movement during daylight and darkness when little is indicated by the stratified net hauls. Pearre (1970) found *Tortanus* to undergo a compact synchronous migration from daytime depths of 6−20 m to the upper 5 m by midnight in Bedford Basin. Bohrer (1980) similarly found that *Tortanus* rose after dusk and remained near the surface until dawn in a 10-m-deep experimental tank. Approximately 30% of the females and 10% of the males were feeding throughout the 24 h with the exception that deep-living males were not feeding during daylight (Table 2).

Centropages hamatus

Centropages was part of the multispecies patch referred to previously which entered our study area around midnight and lasted until dawn (Fig. 6). Both sexes concentrated in the upper 15 m during daylight with part of the population rising to the surface before dusk. This was followed by a general dispersal of the population throughout the upper 20 m during the night. In Oslo fjord, adults and copepodids of *C. hamatus* occurred near the discontinuity layer (~10 m depth) during daytime in June but rose to the surface at night (Hansen 1951). Our traps only captured individuals swimming upward in the water column. Earlier experimental work by Hardy and Bainbridge (1954) found that *Centropages* would undergo sustained upward migration even in strong light when most zooplankters descend. The least activity recorded by the traps was between 17:00 and 21:00 (Fig. 6).

Female *Centropages* fed throughout the 24-h period with the greatest percentage of the population (90−95%) feeding during dusk and dark periods (Table 2). A smaller proportion of the males were feeding at any time with a maximum of ~70% having fed during dusk, dark, and dawn periods. Nicolajsen et al. (1983) studied the feeding periodicity of *C. hamatus* over four 24-h periods in the Øresund between February 17 and May 27 and found that the most intense feeding occurred after dark except on April 7 when midday feeding dominated. Head et al. (1984), using fluorescence techniques, observed maximum feeding in *C. hamatus* between midnight and 06:00 in July in St. Georges Bay.

Calanus finmarchicus

Calanus stages IV and V coexisted in the cold lower layer, below 25 m depth, although they were also present in small numbers all the way to the surface, both day and night (Fig. 7). Part of the population appears to undergo a synchronized migration into the upper mixed layer at dusk (population median depth 12.5 m) but descended below the mixed layer again before midnight. Earlier works reported similar brief dusk ascents followed by either a midnight descent or nighttime dispersal throughout the water column (Marshall and Orr 1955). An analogous situation to St. Georges Bay occurs in Bedford Basin, where *Calanus* progressively retreats to deeper water with a gradual reduction of daily vertical range throughout the summer, as the surface layer warms and deepens (Atherton 1979).

The traps recorded vertical movement in both directions with a major component moving down at 28 m depth (Fig. 7). Surprisingly the dusk ascent, inferred above from the serial horizontal tow results, is not apparent from the trap results. Unfortunately, the traps were not deployed during the major descent of the population. Only four *Calanus* were trapped during the night period, which is consistent with our inter-

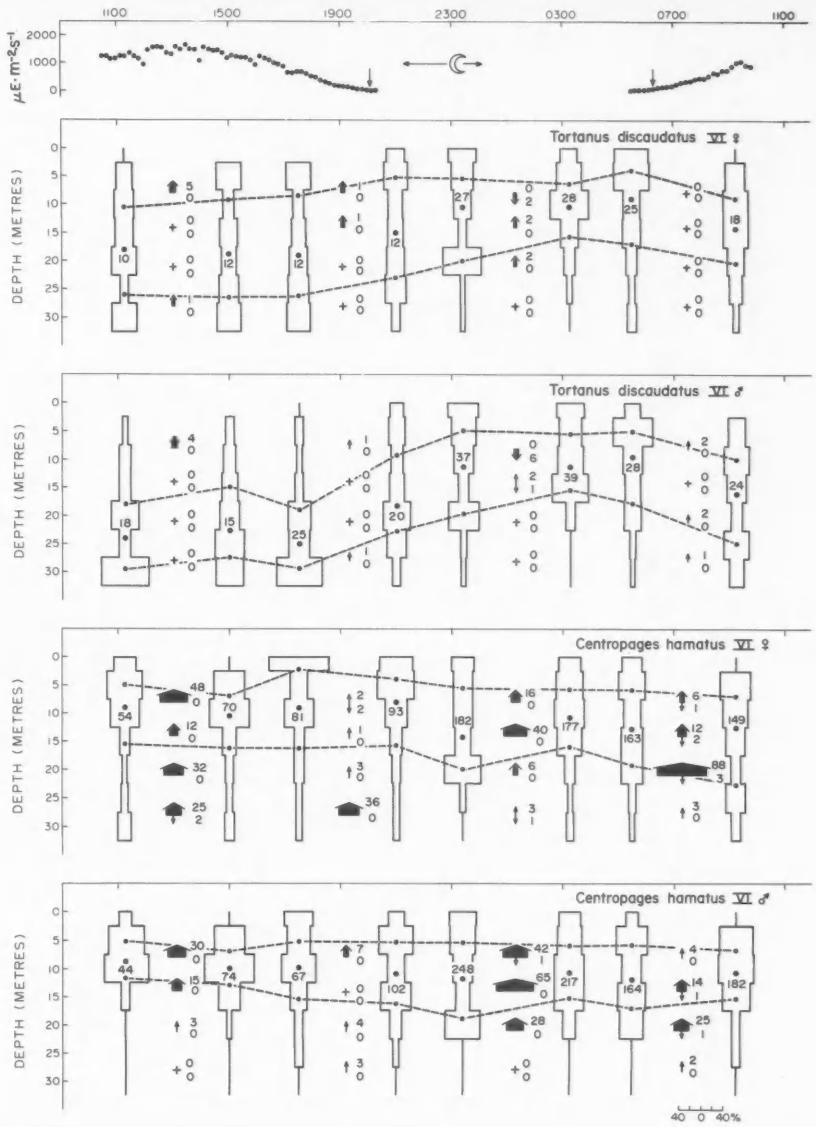

FIG. 6. Depth–frequency diagrams and trap results of *Tortanus discaudatus* females and males and *Centropages hamatus* females and males during the 24-h study (see Fig. 3 for explanation).

pretation that the synchronized migrators had descended to the lower water layer by midnight.

Previous studies on the feeding periodicity of *C. finmarchicus* have given conflicting results (Marshall 1924; Wimpenny 1938; Peruyeva 1977). Bohrer (1980) examined the effect of food concentration on the vertical migration of *Calanus* in a tower tank study. At food concentrations of less than 2 mg Chl $a \cdot m^{-3}$, the median abundance of the population reached its shallowest depth (~1.5 m) around midnight. Increasing the food concentration to >2 mg Chl $a \cdot m^{-3}$ caused the population to rise to the surface the first night but offset the timing the second night, such that the highest ascent occurred before dusk. The chlorophyll *a* concentration in St. Georges Bay is comparable with the lowest values used in Bohrer's experiments which indicates that there must have been another factor inhibiting the upward noctural movement of *Calanus* in the Bay.

The presence of small numbers of *Calanus* in the warm upper layer during daylight and their upward and downward movement into the traps could be interpreted as a continual asynchronous migration by a part of the population. However, none of the *Calanus* captured in the warm waters during daylight contained food in their guts. This suggests that either unhealthy individuals behaved abnormally or hungry individuals were forced to migrate prematurely into the upper layer where they would presumably experience a greater probability of capture by visual predators. It is also possible that two physiologically distinct cohorts are present with different tem-

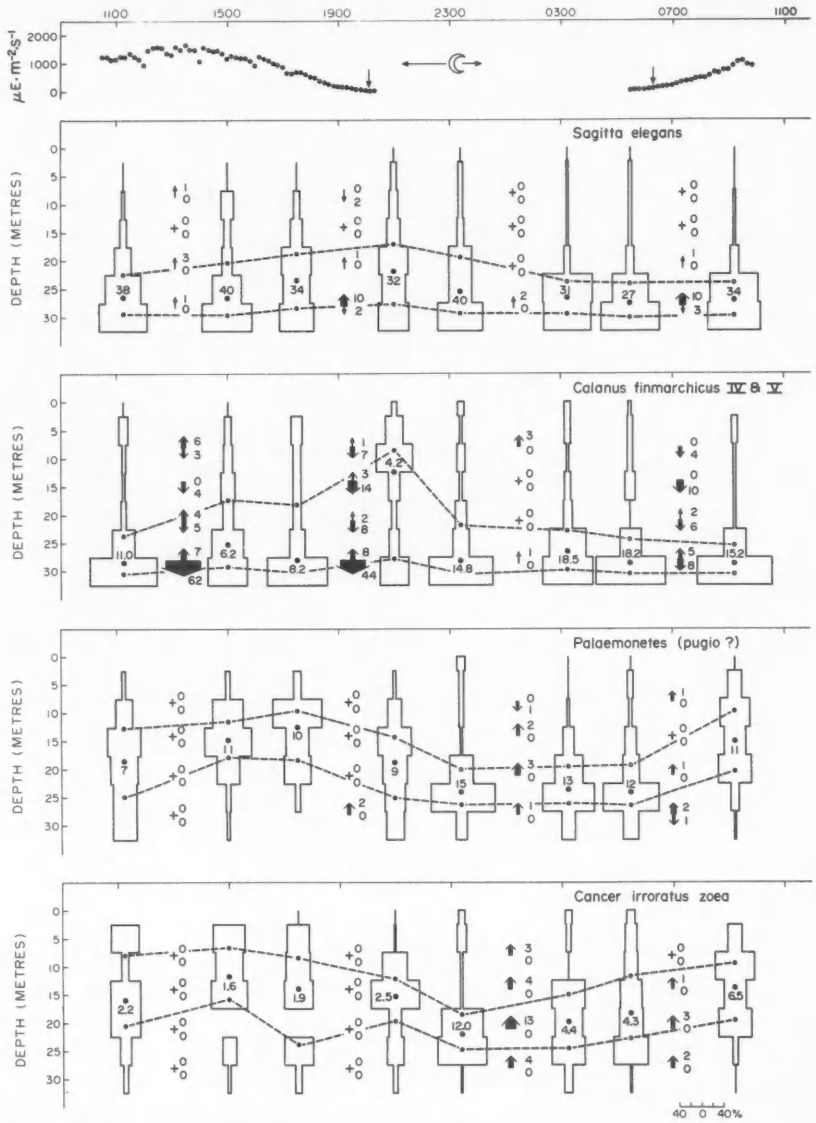

FIG. 7. Depth–frequency diagrams and trap results of *Sagitta elegans*, *Calanus finmarchicus* stages IV and V, *Palaemonetes* (*pugio?*), and *Cancer irroratus* zoea during the 24-h study (see Fig. 3 for explanation).

perature preferences, as suggested by Russell (1934) for the Plymouth area, U.K. If more than one cohort exists in St. Georges Bay, the cohort seeking cold water by day is by far the most abundant and successful. During dusk and dark periods, 44% of the population was observed with accumulated food in their guts, which confirms that the observed synchronous dusk movement was a feeding migration.

Sagitta elegans

The average abundance of this species throughout the water column is remarkably constant over the 24-h study period, ranging between 30 and 40 individuals $\cdot m^{-3}$. This suggests that our deepest tow adequately sampled the near-bottom waters. *Sagitta*, in the size range 3–12 mm total length, frequented the cold bottom water during the day, with a gradual rise in the population in the late afternoon, reaching an upper median population depth of 22 m shortly after sunset (Fig. 7). Thereafter, 50 and 75% of the population returned to depths of 25 m or deeper by midnight and 03:00, respectively. Atherton (1979) described dusk migrations of *S. elegans* into the surface water of Bedford Basin during the summer months, and Lacroix and Morisset (1962) also reported young stages of *S. elegans* moving into the surface water of Baie de Chaleur, P.Q., at night. Trap contents indicate that an upward migration occurred at dusk but no return migration was observed at midnight. As with many of the species examined in this study, the

traps are effective in capturing up-swimming but not down-swimming individuals. Hardy and Bainbridge (1954) observed sustained up-swimming by *Sagitta* in their experimental plankton wheel but only brief periods of active descent.

In St. Georges Bay, 25–35% of the individuals had food in their guts during dusk and night periods which was more than twice the proportion feeding during the rest of the day (Table 2). Previous accounts on the 24-h feeding activity of *S. elegans* also reported that a greater proportion of the population fed after dark (Wimpenny 1938; Rakusa-Suszczewski 1969; Pearre 1973; Feigenbaum 1982). Laboratory studies on the feeding of various *Sagitta* species support these findings (Parry 1944; Reeve 1964). The maximum feeding of *S. elegans* coincides with the upward migration of this species into the upper warm layer in St. Georges Bay.

Cancer irroratus

Cancer zoea appeared to be part of the multispecies patch which passed through the sampling site. *Cancer* was equally abundant down to 20 m depth throughout the day, migrating downwards after dusk with 75% of the population occurring below 15 m by midnight. This was followed by a return migration bringing the population back to daytime depths by 09:00 (Fig. 7). Evidence for diel vertical migrations in nature by brachyuran larvae is lacking (Forward 1977; Sulkin 1984). Forward (1974, 1977) tested the phototactic responses of eight Brachyuran stage I zoea in the laboratory and found that seven species showed positive phototaxis to high light intensities and negative phototaxis to low intensities. This led Forward to predict a vertical migration pattern similar to the one we have found for *C. irroratus* in St. Georges Bay. The sample size was too small for any definitive statement about feeding periodicity, but *Cancer* zoea appear to feed more actively during dusk and dark periods (Table 2).

Palaemonetes (*pugio?*)

The average abundance of this species varied between 7 and 15 individuals $\cdot m^{-3}$ over our 24-h study. With the exception of our noon Clark-Bumpus series, *Palaemonetes* larvae resided chiefly in the 10–20 m depth interval during daylight hours (Fig. 7). At dusk, *Palaemonetes* descended, such that 75% of the population were below 20 m depth by midnight and remained below this depth until 05:00. The dawn return to daylight depths occurred relatively abruptly between 05:00 and 09:00. This species was too rare to be caught often by our plankton traps but the results again indicate that individuals were moving widely at times when modal analysis of depth–frequency data implied that most of the population was stationary. The larval *Palaemonetes* population appears to have fed throughout the day and night (60–70%) with the least feeding activity (48%) at dawn (Table 2).

Evaluation of Trapping Approach

Our original intention was to quantitatively compare two methods of measuring vertical migration. However, a multispecies patch entered our sampling region between dusk to dawn when most of the migration occurred (Fig. 3–7). This event unfortunately prevented any direct numerical comparison of net tow and trap results.

Most of the species in St. Georges Bay moved predominantly upward into the traps over the 24-h period (Table 4).

TABLE 4. Percentage of zooplankters collected ascending of the total plankton trap catch (N = sample size).

	%	N
Cancer irroratus	100	30
Podon intermedius	99	260
Centropages hamatus ♂	99	245
Temora longicornis		
♀ (large)	98	5861
♂	97	3517
Centropages hamatus ♀	97	344
Tautogolabrus adspersus	96	27
Temora longicornis ♀ (small)	92	172
Palaemonetes (pugio)?	86	14
Tortanus discaudatus ♀	86	14
Sagitta elegans	81	36
Evadne nordmanni	75	61
Pseudocalanus minutus		
V	73	819
♂	71	49
♀	66	474
Tortanus discaudatus ♂	65	20
Bivalve larvae	42	211
Calanus finmarchicus IV, V	19	217

However, it is difficult to evaluate what is an acceptable difference from the expected split of 50:50 moving up and down over a 24-h period. Our traps were sampling the populations during 16 h of the day which means that substantial movements could have been missed during the four 2-h periods between trap deployments. Temporal patchiness of the plankton also could have contributed to the trap bias over 24 h in the upward component of migration. Finally, we have measured a 24% reduction of light penetration immediately beneath our traps (Vass et al. 1981) which could have been enough to stimulate upward movement by planktonic organisms. However, in our darkened-trap experiment only adult *Temora* showed increased upward movement during daylight into darkened traps compared with the regular clear traps (Table 3). This does not explain the trap bias at night when many of the species, including the dominants such as *Temora* and *Centropages*, are most actively swimming up into our traps. Moreover, this is the time when depth–frequency histograms indicate that many species had dispersed to evenly populate the upper 20 m.

It is likely that the various degrees of upward bias we observed with our traps (Table 4) may be due to behavioral characteristics peculiar to each species. Zooplankters which descend into our traps are also those which have been observed to swim predominantly up or down in the water column, such as *C. finmarchicus* (Bainbridge 1952) and *P. minutus* (Anderson 1974). *Centropages hamatus* and *T. longicornis* were rarely trapped descending into our traps but these species are known experimentally to have a strong horizontal component to their travel (Cowles 1983; Anderson 1974). Similarly, Hardy and Bainbridge (1954) found that both *C. finmarchicus* and *S. elegans* would swim down in their experimental plankton wheel for periods up to an hour whereas *T. longicornis* and *Centropages* sp. were not found to descend in the same apparatus for more than a few minutes. Brachyuran zoea were not observed to swim down in the plankton wheel (Hardy and Bainbridge 1954) and *Cancer* zoea were never caught descending into our traps. Thus, zooplankters with a large vertical component to their travels are collected de-

TABLE 5. Percentage of *Pseudocalanus* with food in guts caught in plankton traps (N = number of observation in parentheses).

	Day		Dusk		Night		Dawn	
	Female	V	Female	V	Female	V	Female	V
Moving up	3 (63)	3 (134)	5 (92)	7 (179)	28 (80)	17 (107)	48 (64)	40 (99)
Moving down	9 (11)	20 (15)	20 (25)	21 (29)	46 (13)	15 (13)	41 (110)	28 (162)

scending into our traps whereas those species with equivalent or slightly greater horizontal components are rarely collected descending by our traps. It is likely, then, that the observed upward bias may be behavioral, due to a passive sinking of organisms to depth as opposed to an active ascent. The observed underrepresentation of individuals moving downward in most of the species studied could be explained if initial contact with the pyramid funnel causes an upward avoidance reaction. This is considered likely because passive descent by copepods is tail first (Hardy and Bainbridge 1954).

Another possibility is that the increased swimming activity of some species at certain times of the day, such as reported by Champalbert (1978) for copepods, could result in local random movement of their populations. The trap results would give a false impression of upward movement for these species if they avoided up-facing but not down-facing pyramid traps for the reasons hypothesized above. This may explain why *Temora*, *Podon*, and *Centropages* populations appeared to have a stronger upward component of movement during the day and particularly the night than analysis of the sequential population quartiles would indicate.

Judging each species separately from the depth–frequency histograms (Fig. 3–7), we suggest that *Evadne*, *Pseudocalanus*, and *Tortanus* males may have been adequately sampled by the trapping system (Table 4). The avoidance reaction of bivalve larvae to touch is to sink by closing their valves which may explain the greater number of bivalve larvae trapped going down. *Calanus* is the only other species observed more frequently swimming down than up in the water column (Table 4). *Calanus* is one of the few species reported to undergo sustained downward migration, approximately 3 times as fast as its ability to swim upwards (Hardy and Bainbridge 1954).

The present trap design has failed partially to reach our expectations as a method of detecting and quantifying vertical migrations of planktonic organisms. The ascent of zooplankton in the water column is well represented in our trap collections whereas most species are clearly avoiding the traps on descent. The trap collections demonstrate that large-scale vertical movements could occur at times when the depth–frequency diagrams indicate either migration of the population in the opposite direction or that a static condition existed. The sequential horizontal depth tows enable us to delineate the vertical extent of some of these movements, but only if the migrations of each population are nearly synchronous. The simultaneous use of both net tows and traps, therefore, allows a fuller interpretation of the vertical movements of plankton than can be reached from conventional net towing alone.

Feeding Periodicity and Migration Behavior

Wimpenny (1938) tentatively advanced the hypothesis that a high proportion of zooplankters fed continuously in the presence of abundant phytoplankton, based chiefly on observations of *Paracalanus parvus* and *T. longicornis* in the North Sea. Gauld (1953) postulated, from both seasonal field collections and 24-h feeding studies of *C. finmarchicus*, that zooplankters fed continuously if sufficient food was available. He then proposed that the earlier observations that a greater proportion of zooplankters fed at night (Marshall 1924; Wimpenny 1938) were simply a result of more individuals moving into and therefore feeding in the surface, food-rich zone at night. Petipa (1958) observed continuous feeding in *Acartia clausi* adults and copepodids in the Black Sea but also found that the feeding rate changed such that there was a noon and nighttime peak in feeding intensity. Mackas and Bohrer (1976) reported cessation of feeding by *A. clausi* upon satiation, when placed in abundant food. *Calanus* stages IV and V and *Sagitta* both inhabited the cool bottom layer in St Georges Bay during daylight and migrated through the thermocline at dusk to feed. However, *Pseudocalanus* females and stage V, *Podon*, *Evadne*, adult *Centropages*, and *Cancer* zoea all had a greater proportion of individuals with food in their guts at night yet they were all present in the productive upper layer throughout the day. Feeding was definitely intermittent and associated with the migrational behavior in these species but not restricted at any time by the lack of food.

Gauld (1953) observed that North Sea *Calanus* was sometimes distributed throughout the entire water column with no evidence for migratory behavior, yet a certain percentage of the animals at deepest depths always contained food, albeit less than in the more surface collections. He suggested that this population could have been in a continual state of flux with some individuals moving upwards to feed, becoming satiated, and returning to depth, thus explaining not only the lower proportion of individuals feeding but also the presence of animals with less full guts at depth. Pearre (1973) studied a population of *S. elegans* in Bedford Basin and found that he could use surface-living copepods in the guts of the chaetognaths as indicators of the water depth at which they had most recently fed. Similarly, Mackas and Bohrer (1976) used the presence of chlorophyll derivatives in the guts as indicators of recent, near-surface feeding in *Pseudocalanus* collected deep in Bedford Basin. If Gauld's hypothesis of unsynchronized feeding and vertical migration applies to any of the planktonic species in St. Georges Bay, the gut contents of individuals caught in up- and down-facing traps should reveal a greater proportion of individuals having fed swimming down. In our study, only *Pseudocalanus* is both reasonably abundant and roughly approximates at 50:50 split in the up- and down-facing trap catch (Fig. 3; Table 4). After averaging the entire trap catch of females and stage V copepodids, a greater proportion of the *Pseudocalanus* population swimming down was found to have fed compared with that swimming up over the day, dusk, and night sampling periods (Table 5). During the dawn descent of the population the converse occurred. The trap results, therefore, can be used as supportive evidence for some asynchronous vertical movement at certain times in the Bay.

TABLE 6. Observed patterns of vertical migration in St. Georges Bay.

Migration pattern	Species	Peak feeding period
A. Ascent towards surface from variable day depth, departure from uppermost level at or before midnight, return towards surface before dawn, followed by descent to day depth in morning (Cushing 1951)	*Pseudocalanus*	
	♀	Night
	♂	—
	V	Dusk and night
B. Similar pattern to A, with dawn rise not observed	Bivalve larvae	Throughout 24 h
C. Similar pattern to A, but midnight sinking results in an even dispersion throughout the upper mixed layer with no distinct predawn rise	*Temora*	
	♀	Throughout 24 h
	♂	Throughout 24 h
	Podon	Dusk and night
D. Dusk rise reaches uppermost level by midnight then population stays up until dawn	*Tortanus*	
	♀	Throughout 24 h
	♂	Dusk to dawn
E. Dusk rise towards the surface but population returns to day depths by midnight	*Calanus* IV, V	Dusk
	Sagitta	Dusk and night
F. Dusk and nighttime dispersion of population throughout upper mixed layer	*Evadne*	Dusk to dawn
	Centropages	
	♀	Dusk and night
	♂	Dusk to dawn
	Tautogolabrus	Day
G. Dusk sinking to nighttime depths	*Palaemonetes*	Throughout 24 h
	Cancer	Dusk to dawn

Patterns of Vertical Migration

Cushing (1951) reviewed the early literature on the vertical migration of various planktonic organisms and concluded with the following general pattern. Plankters generally ascend towards, but not necessarily to, the surface from a variable day depth. The depths inhabited vary between species and developmental stage and also depend on season and location, with all the variables that introduces. Plankters depart from their shallowest depths at or before midnight and return towards the surface just before dawn, after which a sharp descent occurs to the day depth as the sun rises.

In Table 6 we attempt to classify our observations on the vertical migration of each species in St. Georges Bay. A continuum of patterns exists from Cushing's dusk and dawn rises between "midnight sinking" (type A) to the so-called reverse or nocturnal migration occasionally reported in the literature, where the night level inhabited is deeper than the day depth (types F and G). Bivalve larvae (type B), *Temora* (♀,♂), and *Podon* appear not to return towards the surface at dawn, and the midnight sinking of *Temora* and *Podon* is better described as a dispersion of individuals throughout the upper mixed layer (type C). *Tortanus* (♀,♂) simply ascends at dusk, reaching its uppermost level by midnight, and remains up until the dawn descent (type D). *Calanus* (IV and V) and *Sagitta* migrate up into the mixed layer at dusk but return to the cold bottom layer before midnight (type E). *Centropages* (♀,♂), *Evadne*, and *Tautogolabrus* all disperse, from a relatively shallow day depth, throughout the upper mixed layer at dusk and return at dawn (type F). *Palaemonetes* and *Cancer* also descend at dusk but their populations always stay within narrow depth limits (type G). It is possible that some of our classifications are artificial because of the sampling scheme adopted. For example, a dawn rise of bivalve larvae (type B) may have been missed if our sampling times were slightly too early or late. However, differences such as midnight dispersion (types C and F) and "sinking" (types A, B, and G) are sufficiently distinctive to be thought real.

The advantages of each migration pattern are not clear. Only two of the species studied migrate up from a cool water mass to the warm mixed layer and these are both relics of the winter and spring plankton when the water column is cool and homogeneous. It is difficult to accept that these species have evolved this migration behavior to gain an energy bonus for reproduction and growth through reduced metabolic costs in cold water (as proposed by McLaren 1963, 1974) when these same species undergo an extensive migration earlier in the year under isothermal conditions. The advantages of migrating are more likely related to competition for food (Lane 1975) and avoidance of predators (Murray and Hjort 1912; Stich and Lampert 1981). During our field experiment, phytoplankton production occurred mainly in the upper 20 m with a maximum between 5 and 15 m depth (Fig. 2). Not surprisingly, the bulk of the herbivore and omnivore populations in St. Georges Bay, except *Calanus*, frequent the upper 20 m and it is probable that the different migration patterns and temporal depth preferences help to lessen competition for food (Lane 1975; Hu 1978; Boyd and Smith 1980). Past studies have shown that everything from carbon to inorganic nutrients is rapidly recycled in the upper mixed layer of the Bay during summer (Hargrave et al. 1985). In the present study this is reflected by the lack of a diel change in phytoplankton standing stocks, expressed as chlorophyll *a* and phaeophytin (Fig. 2). *Pseudocalanus* and bivalve larvae probably descend in the water column after feeding in surface waters in early evening to avoid nocturnal predators such as *Tortanus*. Many of the herbivore populations disperse over a broader depth range during the night (types C and F). This behavior may also have evolved to reduce mortality by presenting less dense aggregations to vertically migrating predators. Ohman et al. (1983) observed *Pseudocalanus* sp. to descend in the water column at night presumably to reduce contact with tactile invertebrate predators which inhabit surface

waters at night. They applied their data to McLaren's (1974) demographic model to predict an evolutionary advantage for migrant over nonmigrant populations if this behavior reduced mortality by >16%.

The sightless predators, such as *Tortanus, Sagitta*, and perhaps *Calanus*, all frequent deeper waters during the day presumably because they are more vulnerable themselves to visual predation. Both *Sagitta* and *Calanus* migrate up briefly to feed at dusk; *Tortanus*, however, inhabits a broader depth range both day and night and appears to feed throughout the diel cycle. Conversely, species which use vision to capture prey, such as larval cunner, *Palaemonetes*, and *Cancer*, frequent at least the lower limit of the herbivore zone by day and descend by nightfall. The cunner feeds only during daylight in near-surface waters. These predators must gain some advantage from descending to greater depths at night other than following their prey because their nighttime depth is below most of the herbivore population. This behavior may serve to remove them from predation by jellyfish (*Cyanea*) near the surface at night. In general, then, the diel patterns of zooplankton depth distribution in St. Georges Bay probably reflect a "trade-off" between finding sufficient food and avoidance of being consumed themselves.

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Régime alimentaire et principaux facteurs influençant l'alimentation des larves de capelan (*Mallotus villosus*), d'éperlan (*Osmerus mordax*) et de hareng (*Clupea harengus harengus*) dans un estuaire partiellement mélangé[1]

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Courtois, R. et J. J. Dodson. 1986. Régime alimentaire et principaux facteurs influençant l'alimentation des larves de capelan (*Mallotus villosus*), d'éperlan (*Osmerus mordax*) et de hareng (*Clupea harengus harengus*) dans un estuaire partiellement mélangé. Can. J. Fish. Aquat. Sci. 43: 968–979.

Le régime alimentaire et les rythmes d'alimentation des larves de capelan (*Mallotus villosus*), d'éperlan (*Osmerus mordax*) et de hareng (*Clupea harengus harengus*) ont été étudiés dans le cadre d'un travail visant à déterminer les interactions compétitives potentielles chez ces trois espèces. L'analyse des contenus stomacaux a montré que les larves de capelan de l'estuaire moyen du Saint-Laurent commencent à se nourrir avant la résorption du sac vitellin mais que leurs coefficients d'alimentation sont très faibles (<20 %). À cause de leur petite taille, l'alimentation de ces larves est à toutes fins pratiques restreinte aux tintinnides. Les larves des deux autres espèces se nourrissent plus intensément et montrent un régime alimentaire plus varié. Les variations temporelles des coefficients d'alimentation ont permis de mettre en évidence des cycles circadiens chez les trois espèces et des cycles semi-diurnes chez les larves de capelan et de hareng. L'importance de la lumière pour l'alimentation des larves se trouve confirmée par les cycles circadiens de même que par la distribution préférentielle des larves avec contenu stomacal dans la strate superficielle (0–20 m) de la colonne d'eau. L'absence de relation entre les facteurs biotiques (densité des larves) et l'évolution temporelle des coefficients d'alimentation suggèrent que les cycles semi-diurnes sont dus principalement aux changements de température associés à l'advection des masses d'eau aux stations d'échantillonnage. Malgré des réactions similaires à certaines variables environnementales, les larves des trois espèces étudiées ne semblent pas compétitionner entre elles. Quoique présentes aux mêmes stations d'échantillonnage, elles se retrouvent en fait majoritairement dans des masses d'eau distinctes; leur taille moyenne diffère également. Ces caractéristiques les forcent à exploiter des organismes de taille différente et appartenant majoritairement à des groupes taxonomiques différents.

The food and feeding rate periodicity of larvae of capelin (*Mallotus villosus*), smelt (*Osmerus mordax*), and herring (*Clupea harengus harengus*) were studied to determine the existence of potential interactions between the larvae of these three species. Analyses of the gut contents showed that larvae of capelin started to feed before yolk sac absorption but that the feeding rate was low (<20%) in the lower St. Lawrence estuary; the small size of capelin larvae restricted their feeding to tintinnids. Larvae of the two other species feed at a greater rate than capelin larvae and their food is more diverse. The temporal sequence of feeding incidences showed daily cycles for the three species and semidiurnal cycles for larvae of capelin and herring. The importance of light for feeding of larvae was confirmed by the daily cycles and by the greater abundance of larvae with gut contents in surface waters (0–20 m). From the absence of a relation between biotic factors (density of larvae) and the temporal evolution of feeding incidences, we suggest that semidiurnal cycles are mainly related to temperature changes associated with advection of water masses at the sampling stations. Even though similar reactions to certain environmental variables were observed, we concluded that interspecific competition is minimal between larvae of the three species. Although present at the same stations, larvae of different species mainly occurred in distinct water masses; they are also mainly of different size. Those characteristics force them to exploit organisms of different size and mainly of different species.

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Une gestion efficace des ressources marines nécessite l'évaluation la plus précise possible de l'état des stocks exploités. De ce fait, les gestionnaires des ressources marines ont toujours été préoccupés par les fluctuations d'abondance des stocks causés par un recrutement inégal et imprévisible. Dès la fin du siècle dernier, Fabre-Domergue et Biétrix (1897) ont observé, en élevage, des mortalités massives de larves de poissons marins après la résorption du sac vitellin. Hjort (1914) appliqua ce concept de « période critique » aux stocks exploités et suggéra que le recrutement dépendait de l'importance de la mortalité larvaire, elle-même fonction de la disponibilité d'une nourriture appropriée lors du début de l'alimentation exogène.

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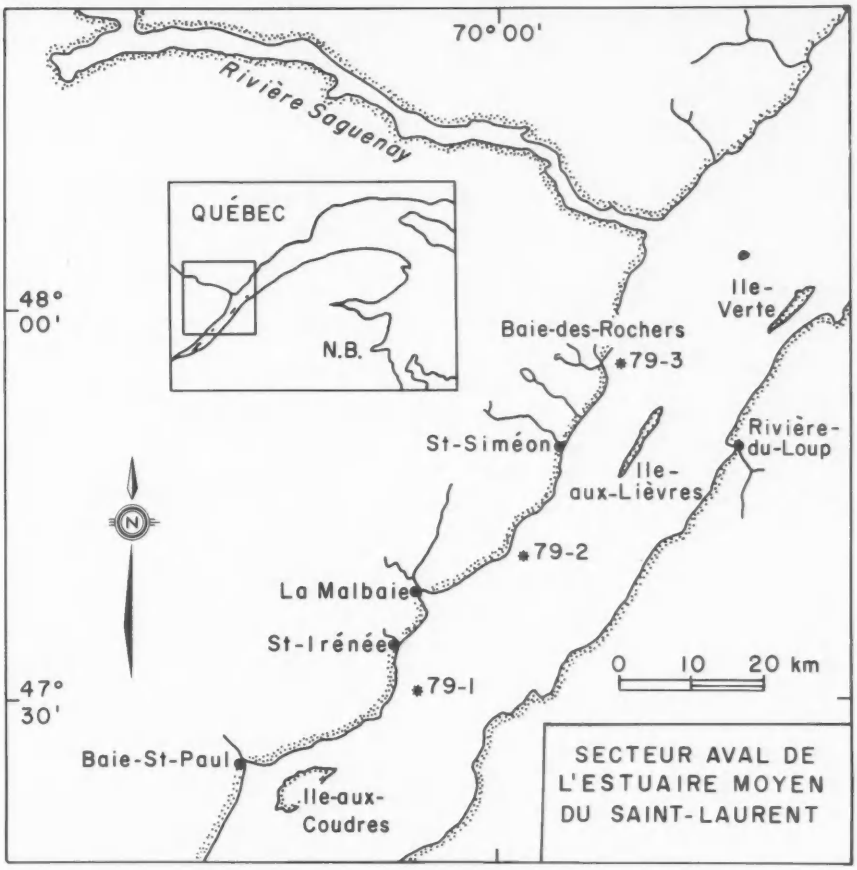

FIG. 1. Secteur aval de l'estuaire moyen du Saint-Laurent, indiquant la position des stations d'échantillonnage en 1979 (79-1 : 47°30,8'N, 70°10,8'W; 79-2 : 47°42,4'N, 69°57,0'W; 79-3 : 47°57,7'N, 69°45,0'W).

D'autres causes ont cependant été suggérées pour expliquer la mortalité larvaire dont, entre autres, la prédation (May 1974; Braum 1978) et la compétition interspécifique lors de la phase larvaire (Isaacs 1963; Lett et Kohler 1976). Cette dernière hypothèse a d'ailleurs été soulevée à maintes reprises lors de colloques internationaux (Blaxter 1974; Hunter 1976; Lasker et Sherman 1981). De plus, la simulation que Lett et Kohler (1976) ont faite tend à démontrer l'influence de l'abondance des larves de maquereau (*Scomber scombus*) sur le taux de croissance du hareng (*Clupea harengus harengus*) du golfe du Saint-Laurent. De façon complémentaire, Laurence *et al.* (1981) ont montré, en condition expérimentale, que la compétition entre les larves de morue (*Gadus morhua*) et d'aiglefin (*Melanogrammus aeglefinus*) mène à des modifications du taux de croissance et de la survie larvaire.

À la suite des travaux de Jacquaz *et al.* (1977) et Able (1978) qui ont montré l'importance de l'estuaire moyen du Saint-Laurent comme site de reproduction et d'élevage pour les larves de capelan (*Mallotus villosus*), d'éperlan (*Osmerus mordax*) et de hareng, il a semblé que ce milieu était un site propice pour l'étude *in situ* des relations entre les larves de ces trois espèces.

La répartition spatio-temporelle des larves de poisson au sein de la communauté planctonique de l'estuaire moyen du Saint-Laurent (Courtois *et al.* 1982) montre toutefois que les larves sont distribuées de façon à minimiser les interactions entre les espèces. Cette séparation spatiale n'est cependant pas absolue; il nous a donc semblé nécessaire d'étudier le régime alimentaire et les facteurs influençant l'alimentation des larves de ces trois espèces afin de vérifier si une compétition alimentaire pouvait exister.

Matériel et méthodes

L'échantillonnage a été effectué à trois stations de l'estuaire moyen du Saint-Laurent (fig. 1). Chaque station a été visitée à la fin des mois de mai, de juin et de juillet 1979 à bord du M/V *Rigolet*, un petit navire océanographique de 19,8 m.

À chacune des stations, un prélèvement oblique d'environ 10 min a été effecuté dans trois strates (0–20, 20–40 et 40–60 m), à toutes les 3 h, pendant 48 h. Avant chaque série de traits, le navire était ancré à une bouée indiquant l'emplacement de la station; des données de température, de conductivité, de vitesse et de direction des courants étaient alors enre-

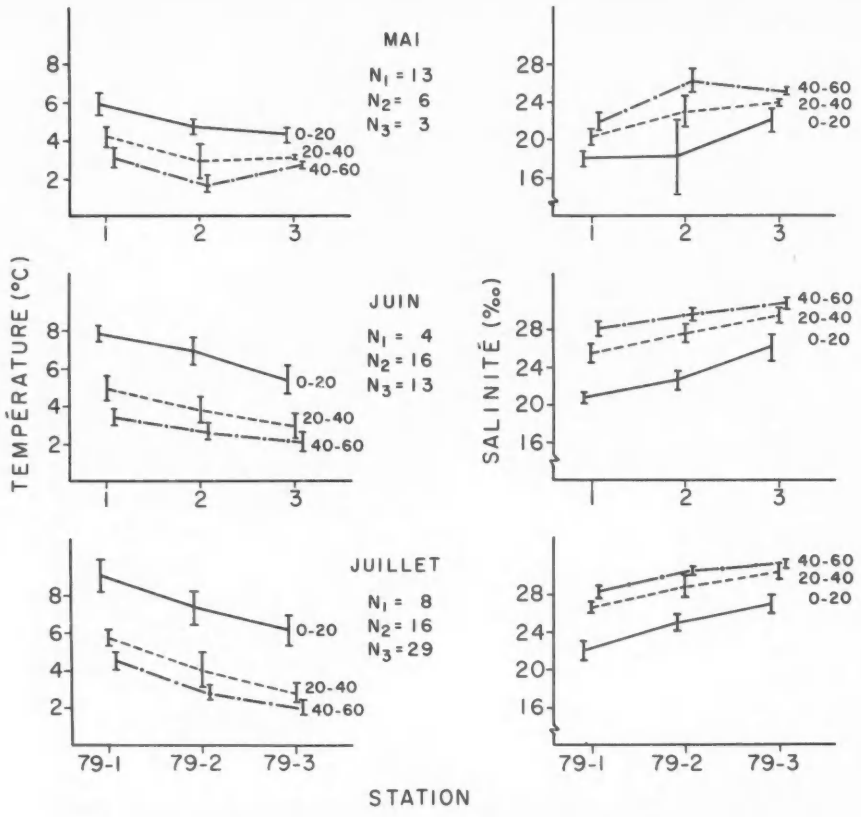

FIG. 2. Température et salinité moyennes des masses d'eau échantillonnées aux stations 79-1, 79-2 et 79-3 en mai, juin et juillet 1979. Les traits verticaux représentent l'écart-type des valeurs. N_1, N_2 et N_3 = nombre d'observations pour les stations 79-1, 79-2 et 79-3 respectivement.

gistrées sur toute la colonne d'eau par palier de 5 m à l'aide d'un courantomètre Aanderaa muni de senseurs STP. De plus, la température de surface et la valeur de l'indice de Secchi étaient notées. La salinité et la densité ont été calculées à l'aide des données de température et de conductivité. Les données physiques ont été complétées par la hauteur de la marée tirée des tables de marée, un indice d'éclairement (0 = nuit; 1 = pénombre (de 4 h à 6 h et de 20 h 30 à 22 h 30); 3 = jour) et un indice de stratification ($\Delta\sigma_t$ 5 − 35) :

$$\Delta\sigma_t\ 5 - 35 = \frac{\Delta\sigma_t}{\Delta z}$$

où $\Delta\sigma_t$ = densité à 5 m − densité à 35 m et Δz = intervalle de profondeur.

L'échantillonnage a été réalisé à l'aide d'un échantillonneur Bongo de 50 cm muni d'un débitmètre et d'un système d'ouverture et de fermeture (McGowan et Brown 1966). La profondeur des traits était enregistrée par une sonde TDR (time depth recorder) et vérifiée à bord du navire lors de l'échantillonnage grâce à un télémètre Furuno monté sur le câble hydrologique. De plus amples détails des techniques utilisées sont disponibles dans Courtois *et al.* (1982) et Fortier et Leggett (1982).

Les échantillons ont été préservés dans du formol 4 % tamponné au borate de sodium. Le tri a été effectué au laboratoire et les larves d'éperlan et de hareng, habituellement de taille supérieure à 7 mm, ont été triées à partir de l'échantillon complet. Un partiteur Folsom a été utilisé pour dénombrer les larves de capelan dans les échantillons qui semblaient contenir plus de 200 larves.

L'examen des contenus stomacaux a été fait à la loupe binoculaire en éclairage diascopique et à un grossissement de 25 ou 50×, sauf pour les très petites proies où un grossissement de 100× a parfois été nécessaire. Les larves ont été examinées une à une en les étalant sur une lame de microscope. Les proies étaient habituellement visibles à travers la paroi intestinale des larves et apparaissaient comme une tache sombre. Elles étaient alors retirées pour identification en disséquant délicatement le tube digestif des larves à l'aide de fines aiguilles entomologiques montées sur des bâtonnets. Les larves plus grandes que 15 mm sont généralement plus opaques; un trempage d'environ 10 s dans du KOH 5 % permettait alors de les clarifier suffisamment pour le repérage des proies. Par contre, le tube digestif des spécimens les plus opaques était systématiquement disséqué. La longueur standard des larves de même que la longueur et la largeur maximums des proies (appendices exclus) ont été mesurées au dixième de millimètre grâce à un oculaire micrométrique.

Certaines larves ont été détériorées lors de l'échantillonnage, mais seuls les spécimens montrant un tube digestif complet ont

été considérés pour le calcul des coefficients d'alimentation (pourcentage de larves dont le tube digestif contient au moins une proie). Généralement, plus de 80 % des larves de capelan et d'éperlan étaient en bon état comparativement à 50 à 70 % des larves de hareng.

Les variations horaires du coefficient d'alimentation sont présentées sous forme de séries filtrées (moyenne mobile $N = 3$; Legendre et Legendre 1979) afin de réduire les bruits causés par les imprécisions d'échantillonnage. De plus, à la station 79−3 en juillet, une donnée manquante a été remplacée par la moyenne des deux points contigus visiblement congruents.

L'effet de la profondeur et de l'éclairement sur l'alimentation des larves a été dégagé à l'aide de tableaux de contingence (Legendre et Legendre 1979). Les tableaux de contingence servent à illustrer le type de relation qui unit deux variables. On indique dans la partie supérieure du tableau la proportion des observations dans chaque classe de la première variable pour l'ensemble des échantillons (probabilités inconditionnelles). Les autres lignes du tableau servent à indiquer les proportions obtenues lorsque l'on tient compte également des différentes classes de la deuxième variable (probabilités conditionnelles). Les probabilités conditionnelles supérieures aux probabilités inconditionnelles montrent l'effet de la seconde variable sur la première lorsque l'indépendance des deux variables a été rejetée à l'aide d'un test du chi carré. L'effet des autres variables biologiques et physiques sur les coefficients d'alimentation a été étudié à l'aide de corrélations non paramétriques de Kendall (Scherrer 1984).

Résultats

Caractéristiques des masses d'eau

Nos stations d'écantillonnage étaient situées dans des secteurs typiques d'un estuaire partiellement mélangé. On y retrouve en surface une couche d'eau saumâtre virtuellement limitée aux 20 premiers mètres de la colonne d'eau et sous laquelle pénètrent les masses d'eau marines (fig. 2).

On note, de la station 1 à la station 3, une diminution de la température moyenne des masses d'eau d'environ 2°C. Ce changement, accompagné d'une augmentation de salinité d'environ 4 ‰, est causé par le mélange progressif des masses d'eau douce coulant en surface avec les eaux marines pénétrant en profondeur. Ce phénomène conduit à une stratification nettement plus faible à la station 79-3 (fig. 3). On retrouve à cette station des conditions presque marines, particulièrement en profondeur, avec des eaux de moins de 3°C et une salinité d'environ 30 ‰. La température de surface et la turbidité sont également plus faibles à cette station. Sur l'échelle temporelle, des modifications sont surtout perceptibles aux deux stations d'amont. Il s'agit principalement d'un réchauffement progressif des masses d'eau d'environ 1,5°C par mois, mais également d'une augmentation de la salinité (≅4 ‰) dès le mois de juin, résultant de la diminution du débit d'eau douce (fig. 2).

Coefficients d'alimentation

Les coefficients d'alimentation observés chez les larves de capelan sont très faibles. Ceci est particulièrement évident en mai, alors qu'à toutes fins pratiques des larves de capelan avec contenu stomacal n'ont été retrouvées qu'à la station 79-3; de plus, à cet endroit, seulement 2,1 % des larves de cette espèce avaient des proies dans l'intestin. En juin et juillet, leur

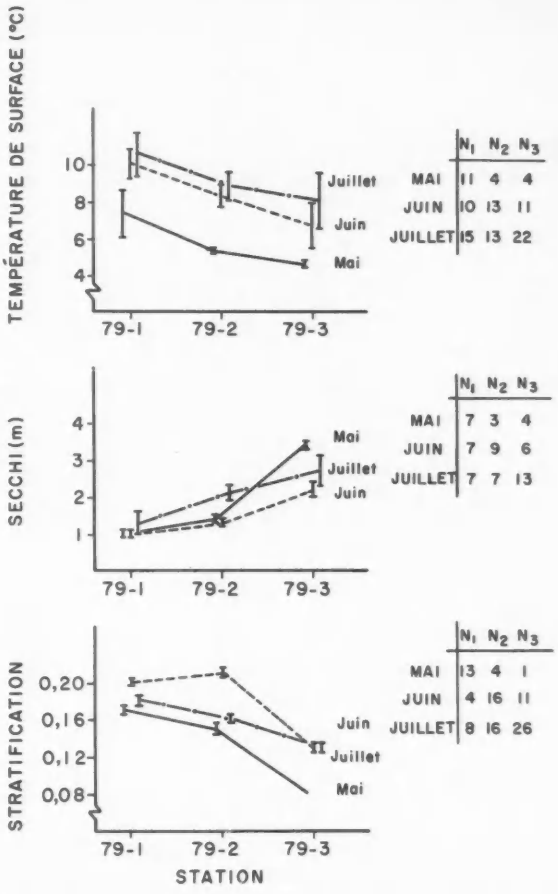

FIG. 3. Moyenne et écart-type des données de température de surface, indice de Secchi et stratification enregistrées en mai, juin et juillet 1979 aux trois stations d'échantillonnage. N_1, N_2 et N_3 représentent le nombre d'observations aux stations 79-1, 79-2 et 79-3 respectivement.

coefficient d'alimentation augmente sensiblement : il est de 4,1−11,0 et 2,3 % aux stations 79-1, 79-2 et 79-3 en juin et passe à 6,1−19,8 et 9,9 % à ces mêmes stations en juillet.

Des larves de capelan plus grandes que 7 mm n'ont été observées qu'à la station 79-3 en juillet; leur coefficient d'alimentation était alors de 12,1 % (tableau 1).

La proportion de larves de capelan avec sac vitellin oscille entre 22,1 et 55,9 %. Les spécimens de cette espèce commencent à se nourrir avant la résorption du sac vitellin, mais le coefficient d'alimentation est toutefois lègèrement supérieur chez les larves dont le sac vitellin est résorbé; ceci est visible pour chaque station et à chaque période d'échantillonnage où des larves de capelan avec contenu stomacal ont été retrouvées (tableau 1).

L'éperlan n'est bien représenté qu'à la station 79-1 : 87,6 % des captures y ont été enregistrées, en majorité dans les deux premiers mois d'échantillonnage. Le coefficient d'alimentation des larves d'éperlan varie peu de mai à juin (environ 11 %). En juillet, il passe à 27,3 % à la station 79-1, mais seulement 11 spécimens ont été rapportés (tableau 2).

La majorité des larves d'éperlan échantillonnées avaient

TABLEAU 1. Pourcentage de larves de capelan avec sac vitellin et coefficients d'alimentation observés lors des échantillonnages réalisés dans l'estuaire moyen du Saint-Laurent en 1979. *N* représente le nombre de larves avec tube digestif intact.

			Avec sac vitellin		Sans sac vitellin		Total	
Mois	Station	% avec sac vitellin	*N*	Coefficient (%)	*N*	Coefficient (%)	*N*	Coefficient (%)
Mai	79−1	36,3	647	0,0	1134	0,0	1781	0,0
	79−2	29,0	202	0,0	494	0,2	696	0,1
	79−3	55,9	320	1,9	252	2,4	572	2,1
Juin	79−1	22,1	378	2,6	1332	4,5	1710	4,1
	79−2	27,9	814	9,7	2100	11,5	2914	11,0
	79−3	40,0	1310	1,8	1966	2,6	3276	2,3
Juillet	79−1	48,0	416	5,5	451	6,7	867	6,1
	79−2	45,8	347	18,4	410	21,0	757	19,8
	79−3 (<7 mm)	44,6	925	8,3	1150	11,2	2075	9,9
	(>7 mm)	0,0	—	—	33	12,1	33	12,1

TABLEAU 2. Pourcentage de larves d'éperlan avec sac vitellin et coefficients d'alimentation observés lors des échantillonnages réalisés dans l'estuaire moyen du Saint-Laurent en 1979. *N* représente le nombre de larves avec tube digestif intact.

			Avec sac vitellin		Sans sac vitellin		Total	
Mois	Station	% avec sac vitellin	*N*	Coefficient (%)	*N*	Coefficient (%)	*N*	Coefficient (%)
Mai	79−1	32,9	53	9,4	108	4,6	161	12,4
	79−2	0,0	—	—	2	0,0	2	0,0
	79−3	—	—	—	—	—	—	—
Juin	79−1	0,0	—	—	289	11,8	289	11,8
	79−2	2,9	1	0,0	33	12,1	34	11,8
	79−3	0,0	—	—	28	10,7	28	10,7
Juillet	79−1	0,0	—	—	11	27,3	11	27,3
	79−2	0,0	—	—	1	0,0	—	—
	79−3	0,0	—	—	1	0,0	1	0,0

TABLEAU 3. Pourcentage de larves de hareng avec sac vitellin et coefficients d'alimentation observés lors des échantillonnages réalisés dans l'estuaire moyen du Saint-Laurent en 1979. *N* représente le nombre de larves avec tube digestif intact.

			Avec sac vitellin		Sans sac vitellin		Total	
Mois	Station	% avec sac vitellin	*N*	Coefficient (%)	*N*	Coefficient (%)	*N*	Coefficient (%)
Mai	79−1	—	—	—	—	—	—	—
	79−2	—	—	—	—	—	—	—
	79−3	—	—	—	—	—	—	—
Juin	79−1	15,9	7	0,0	37	8,1	44	6,8
	79−2	13,1	143	0,0	949	0,4	1092	0,4
	79−3	29,4	266	2,3	639	6,7	905	5,4
Juillet	79−1	3,0	2	0,0	64	53,1	66	51,5
	79−2	0,0	—	—	115	22,6	115	22,6
	79−3	0,0	—	—	79	39,2	79	39,2

résorbé leur sac vitellin. Cependant, en mai à la station 79-1, 32,9 % des larves de cette espèce étaient vésiculées (tableau 3). Chez ces dernières, 9,4 % possédaient un contenu stomacal, soit pratiquement le double du coefficient d'alimentation des larves d'éperlan sans sac vitellin (tableau 2). Un test du χ^2 démontre toutefois que ces différences de proportion ne sont pas significatives ($\chi^2 = 0{,}71$, $p > 0{,}05$).

Les jeunes larves de hareng sont apparues en juin et étaient alors surtout concentrées en aval (stations 79-2 et 79-3). Les coefficients d'alimentation de ces dernières varient passablement d'une station à l'autre et d'un mois à l'autre. En juin, le plus petit coefficient (0,4 %) est enregistré à la station 79-2

TABLEAU 4. Comparaison de la distribution verticale des larves de capelan, d'éperlan et de hareng avec contenu (cont) et sans contenu stomacal (scont). Probabilités inconditionnelles : proportion de larves avec contenu et sans contenu stomacal dans l'ensemble des échantillons; probabilités conditionnelles : proportions de larves avec et sans contenu stomacal dans chaque strate échantillonnée. Les probabilités conditionnelles supérieures aux probabilités inconditionnelles montrent l'effet de la profondeur sur la distribution des larves.

	Capelan[a]		Éperlan[b]		Hareng[c]	
	Cont	Scont	Cont	Scont	Cont	Scont
Probabilités inconditionnelles	0,07	0,93	0,12	0,88	0,05	0,95
Probabilités conditionnelles						
0–20 m	0,08	0,92	0,15	0,85	0,14	0,86
20–40 m	0,06	0,94	0,10	0,90	0,03	0,97
40–60 m	0,05	0,95	0,09	0,91	0,03	0,96
	$\chi^2 = 20,13$ $p < 0,001$ $N = 9524$		$\chi^2 = 3,17$ $p > 0,05$ $N = 450$		$\chi^2 = 91,40$ $p < 0,001$ $N = 2222$	

[a]Stations 79–1, 79–2 et 79–3 en juin et stations 79–1 et 79–2 en juillet.
[b]Station 79–1 en mai et juin.
[c]Stations 79–2 et 79–3 en juin et stations 79–1 et 79–2 en juillet.

TABLEAU 5. Analyse de la répartition des larves avec contenu stomacal selon la profondeur et l'éclairement. Les stations d'échantillonnage considérées sont les mêmes qu'au tableau 4. Probabilités inconditionnelles : proportion de larves avec contenu stomacal à chaque strate échantillonnée; probabilités conditionnelles : proportion de larves avec contenu stomacal à 20, 40 et 60 m selon l'éclairement.

	Capelan			Éperlan		Hareng		
	20	40	60	20	40–60	20	40	60
Probabilités inconditionnelles	0,61	0,25	0,14	0,83	0,17	0,54	0,22	0,25
Probabilités conditionnelles								
Jour	0,67	0,21	0,12	0,90	0,10	0,72	0,18	0,10
Pénombre	0,53	0,39	0,08	0,77	0,23	0,38	0,15	0,46
Nuit	0,47	0,27	0,26			0,24	0,32	0,44
	$\chi^2 = 36,6$ $p < 0,001$ $N = 664$			$\chi^2 = 5,06$ $0,01 < p < 0,05$ $N = 54$		$\chi^2 = 26,01$ $p < 0,001$ $N = 114$		

alors qu'aux stations 79-1 et 79-3, les larves de hareng montrent des taux de 8,4 et 5,4 % respectivement (tableau 3). En juillet, les coefficients d'alimentation augmentent considérablement; ils sont de 51,6, 22,6 et 39,2 % aux stations 79-1, 79-2 et 79-3 respectivement.

Les larves de hareng avec sac vitellin sont à toutes fins pratiques restreintes au mois de juin. Durant ce mois, elles représentent plus de 29 % des larves de hareng capturées à la station 79-3 mais moins de 16 % du total capturé aux deux autres stations (tableau 3). Contrairement aux larves de capelan et d'éperlan, les larves de hareng ne se nourrissent généralement pas avant la résorption du sac vitellin. Des écarts à cette règle n'ont été remarqués qu'à la station 79-3 en juin, alors que 2,3 % des larves de hareng vésiculées possédaient un contenu stomacal (tableau 3).

Distribution verticale et rythmes d'alimentation

Des larves avec contenu stomacal ont été prélevées jusqu'à des profondeurs de 60 m et ce, pour les trois espèces étudiées. L'analyse par tableau de contingence montre cependant qu'une plus forte proportion de larves de capelan et de hareng avec contenu stomacal sont échantillonnées en surface (0–20 m) alors que celles sans contenu se retrouvent en plus grande proportion à plus de 20 m ($p < 0,001$; tableau 4). Le coefficient d'alimentation des larves de capelan est de 8 % en surface comparativement à 6 et 5 % à 40 et 60 m. Les larves de hareng montrent quant à elles un taux de 14 % dans la strate 0–20 m, mais de seulement 3 % à de plus grandes profondeurs (tableau 4). Par contre, l'influence de la profondeur sur l'alimentation des larves d'éperlan n'a pas été révélée par cette analyse ($p > 0,05$; tableau 4).

L'effet combiné de la profondeur et de l'éclairement pour l'alimentation des larves de poissons est également démontré par l'analyse des tableaux de contingence. La majeure partie des larves avec contenu stomacal est notée le jour dans la partie supérieure de la colonne d'eau. En pénombre et la nuit, la proportion de larves avec contenu stomacal diminue en surface, mais augmente parfois considérablement, à plus de 20 m. Ce phénomène est mis en évidence pour les trois espèces étudiées bien qu'il ait fallu regrouper les données des classes extrêmes pour satisfaire les postulats de base du χ^2 dans le cas des larves d'éperlan (tableau 5).

TABLEAU 6. Analyse de la répartition des larves sans contenu stomacal selon la profondeur et l'éclairement. Les stations d'échantillonnage considérées sont les mêmes qu'au tableau 4. Probabilités inconditionnelles : proportion de larves selon la profondeur; probabilités conditionnelles : proportion de larves selon la profondeur et l'éclairement.

	Capelan			Éperlan		Hareng		
	20	40	60	20	40–60	20	40	60
Probabilités inconditionnelles	0,53	0,28	0,19	0,38	0,62	0,17	0,39	0,43
Probabilités conditionnelles								
Jour	0,54	0,28	0,18	0,37	0,63	0,16	0,40	0,45
Pénombre	0,52	0,25	0,24	0,38	0,62	0,28	0,44	0,29
Nuit	0,50	0,29	0,21			0,14	0,36	0,51
	$\chi^2 = 32.5$ $p < 0,001$ $N = 8860$			$\chi^2 = 0,12$ $p > 0,05$ $N = 396$		$\chi^2 = 54,00$ $p < 0,001$ $N = 2108$		

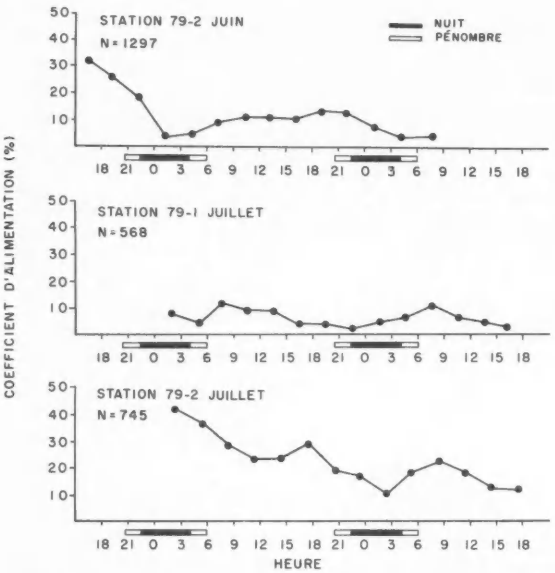

FIG. 4. Évolution du coefficient d'alimentation des larves de capelan aux stations présentant les plus longues séries temporelles (strate 0–20 m). Moyennes mobiles sur trois valeurs successives. *N* représente le nombre de larves examinées.

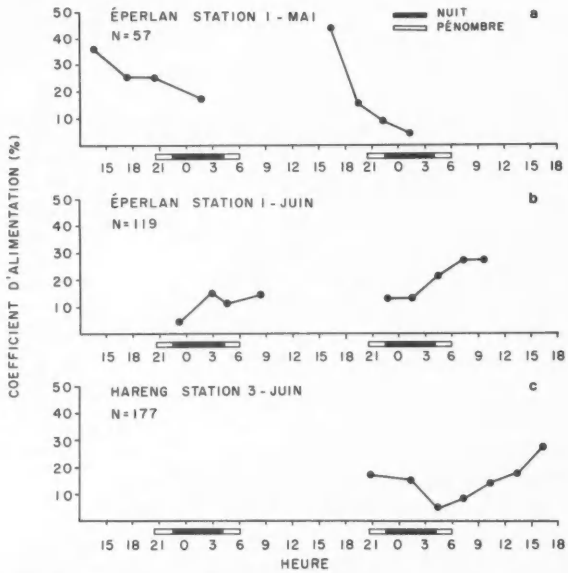

FIG. 5. Évolution du coefficient d'alimentation des larves d'éperlan (a et b) et de hareng (c) échantillonnées en surface (0–20 m) aux stations présentant les plus longues séries temporelles. Moyennes mobiles sur trois valeurs successives.

L'influence de l'éclairement se manifeste également sur les larves de capelan et de hareng sans contenu stomacal (tableau 6), quoique de façon moins nette que pour les larves avec contenu stomacal. Chez les larves d'éperlan, l'effet de la luminosité n'a pu être démontré que pour celles avec contenu stomacal (tableaux 5 et 6).

L'évolution du coefficient d'alimentation en fonction de l'heure d'échantillonnage montre que l'alimentation des larves des trois espèces étudiées s'initie dès l'aube et se poursuit jusqu'au crépuscule (fig. 4, 5 et 6). Pour les larves de capelan, les coefficients d'alimentation maximums sont notés durant le jour mais peuvent se situer entre 7 et 19 h selon la station considérée. En outre, le rythme d'alimentation des larves de capelan de la station 2 en juillet décroît de façon linéaire ($y = 36,12 - 0,61x$; $r = -0,85$; $p < 0,01$) au cours de la période d'échantillonnage (fig. 4).

Les séries obtenues pour les larves d'éperlan et de hareng, quoique moins précises à cause de leur plus faible abondance, montrent que ces deux espèces réagissent de façon similaire aux larves de capelan. Chez l'éperlan, les coefficients sont maximum en surface (0–20 m) durant le jour et paraissent décliner à partir de 15 ou 16 h (fig. 5a); leur alimentation semble débuter dès l'aube (fig. 5b). Pour le hareng, les coefficients d'alimentation augmentent de l'aube jusqu'en fin d'après-midi et déclinent par la suite jusqu'au début du jour suivant (fig. 5c). Ce rythme circadien est encore mieux illustré par l'échantillonnage plus serré réalisé à la station 79-3 en juillet (fig. 6b). Quelques échantillons ne contenaient pas de larves de hareng mais la tendance paraît évidente : l'alimentation débute tôt le matin et atteint son maximum en après-midi.

L'alimentation des larves n'est pas régie exclusivement par la luminosité. Ceci semble démontré par la tendance linéaire et

TABLEAU 7. Corrélation (τ de Kendall) entre les coefficients d'alimentation des larves de capelan et de hareng et les facteurs susceptibles de les influencer. Station 79−3 en juillet; strate superficielle (0−20 m). *0,05 < p < 0,01; **p < 0,01.

	Coefficient d'alimentation			
	Capelan		Hareng	
	τ	N	τ	N
Densité des larves de capelan	−0,18	26	−0,10	22
Densité des larves de hareng	0,18	22	0,12	22
Densité des autres larves	0,07	26	−0,09	22
Densité du microplancton	0,32*	26	0,04	22
Température	0,30*	26	0,23*	22
Salinité	−0,38**	26	−0,16	22
Indice d'éclairement	0,15	26	0,50**	22
Indice de Secchi	−0,03	13	−0,10	13
Température de surface	0,30*	22	0,27*	22
Indice de stratification	0,17	26	0,07	22
Hauteur de la marée	−0.27*	26	−0,09	22

le pic d'alimentation nocturne observés à la station 2 en juillet (fig. 4). Pour le capelan, le coefficient d'alimentation semble dépendre entre autres de l'état de la marée. Ceci est perceptible à la station 3 en juillet où l'échantillonnage horaire a permis l'obtention d'une série plus fine (fig. 6a). À cette station, le rythme circadien du coefficient d'alimentation est évident et il semble que ce coefficient augmente jusqu'aux environs de 13 à 16 h. De plus, les coefficients maximums sont observés à marée basse (fig. 6a). Des corrélations non paramétriques montrent que ce phénomène est lié à l'arrivée de masses d'eau plus chaudes, moins salées et plus riches en microplancton en provenance de l'amont de l'estuaire (tableau 7). La superposition des cycles circadien et semi-diurne crée une augmentation apparente du coefficient d'alimentation au cours de la nuit. Ceci est dû au fait que l'on échantillonne à marée basse des masses d'eau qui offrent des conditions plus favorables à l'alimentation des larves. Pour le hareng, les corrélations significatives notées pour l'indice d'éclairement (τ = 0,50, p < 0,01), la température (τ = 0,23, p < 0,05) et la température de surface (τ = 0,27, p < 0,05) montrent que l'alimentation des larves est non seulement meilleure le jour, mais également dans les masses d'eau provenant de l'amont de l'estuaire (tableau 7).

Régime alimentaire

En mai 1979, seulement 13 larves de capelan et 20 larves d'éperlan avec contenu stomacal ont été recensées; ce faible nombre d'observations rend les résultats de cette période moins fiables. Il semble néanmoins intéressant de mentionner que la majorité (61 %) des larves de capelan avec contenu stomacal avait ingéré l'algue unicellulaire *Coscinodiscus centralis* (tableau 8). Les autres taxons recensés sont les tintinnides (*Tintinnopsis turbo*, 15 %), des œufs de copépodes (25 %), de même qu'un organisme non identifié. Comme la taille des larves de capelan change peu selon la station ou le mois (≅5 mm; tableau 9), leur régime alimentaire reste peu varié : généralement, les tintinnides apparaissent chez plus de 85 % des larves avec contenu stomacal. La station 3 est la seule où d'autres taxons tels *Coscinodiscus centralis* et des œufs d'invertébrés apparaissent en nombre significatif (plus de 5 %).

En mai 1979, les larves d'éperlan se nourrissaient déjà exclusivement de copépodites et de copépodes adultes (tableau 8) malgré leur faible taille (8−9 mm; tableau 9). En juin, l'étendue des tailles est beaucoup plus vaste et le régime alimentaire des larves d'éperlan devient plus varié. Celles-ci continuent à ingérer des copépodites et des copépodes adultes (fréquence d'environ 30 %), mais les nauplii de cirripèdes (*Balanus* sp.) se rencontrent maintenant chez 22 % des larves d'éperlan ayant un contenu stomacal. On note aussi la présence de matière digérée (10 %), d'œufs de copépodes (5 %) et de taxons non identifiés (2 %). Finalement, environ 15 % des larves d'éperlan avec contenu stomacal sont infestés de trématodes alors que 2 % d'entre elles portent des cestodes (tableau 8). La très grande majorité (≅95 %) des copépodes ingérés par les larves d'éperlan appartenait au genre *Eurytemora*.

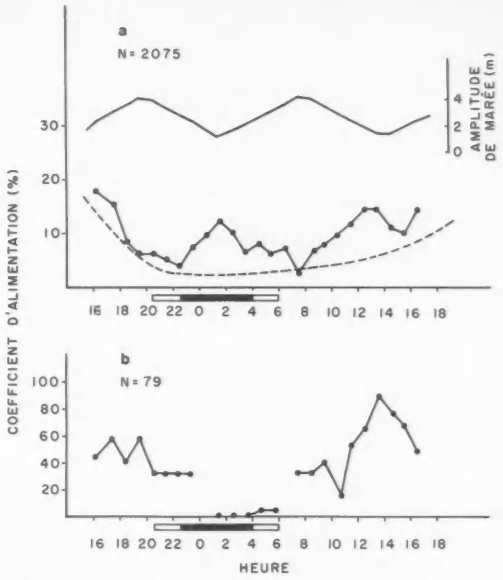

FIG. 6. Relation entre le coefficient d'alimentation et la hauteur de la marée pour les larves de capelan (a) et de hareng (b) échantillonnées en surface (0−20 m) à la station 79-3 en juillet. Moyennes mobiles sur trois valeurs successives. Pour le capelan, une courbe à main levée (traits discontinus) tracée au centre des résidus de la régression du coefficient d'alimentation selon l'amplitude de la marée (y = 15,309 − 2,188; r = 0,54; p < 0,01) met en évidence la tendance circadienne.

Ce sont les larves de hareng qui ont le régime alimentaire le plus varié (tableau 8) : en juin, les jeunes larves ($\overline{LS}$ ≅ 8 mm; tableau 9) mangent principalement la diatomée *Coscinodiscus centralis* (fréquence d'environ 35 %), des tintinnides (22 %) et des œufs de copépodes (47 %). La matière digérée est assez fréquente (31 %); des cestodes ont été retrouvés chez 2 % des spécimens avec contenu stomacal (tableau 8).

En juillet, le régime alimentaire des larves de hareng est essentiellement composé des mêmes proies avec cependant une diminution de l'importance de *C. centralis* accompagnée d'une augmentation notable de l'importance des copépodites et des copépodes adultes. Des parasites sont également retrouvés en plus grand nombre : 30 % des larves de hareng avec contenu stomacal contiennent des cestodes et 9 % d'entre elles contiennent des trématodes (tableau 8).

En juillet, la taille moyenne des larves de hareng avec contenu stomacal est plus élevée à la station 79-1 ($\overline{LS}$ = 16,40 mm)

TABLEAU 8. Indice de fréquence (%) des différentes proies dans les contenus stomacaux des larves de capelan, d'éperlan et de hareng lors de l'échantillonnage réalisé dans l'estuaire moyen du Saint-Laurent en 1979. *N* représente le nombre de larves avec contenu stomacal.

	Capelan			Éperlan			Hareng		
	Mai	Juin	Juillet	Mai	Juin	Juillet	Mai	Juin	Juillet
N	13	498	407	20	41	7	0	55	90
Phytoplancton									
C. centralis	61	1	1	—	2	—	—	35	1
Autres	—	—	—	—	—	—	—	2	—
Protozoaires									
Tintinnopsis sp.	15	90	87	—	—	—	—	22	19
Copépodes									
Oeufs	25	2	6	—	5	—	—	31	47
Nauplii	—	—	—	—	—	—	—	—	—
Copépodites	—	—	—	95	34	—	—	2	19
Copépodes adultes	—	—	—	5	29	71	—	2	22
Autres									
Nauplii de cirripède	—	—	—	—	22	—	—	—	9
Matière digérée	—	9	15	5	10	—	—	31	16
Divers	8	1	8	—	2	—	—	13	2
Parasites									
Cestodes	—	—	—	—	2	57	—	2	30
Trématodes	—	—	—	—	15	29	—	—	9

TABLEAU 9. Longueur standard selon le mois et la station d'échantillonnage pour les larves de capelan, d'éperlan et de hareng avec contenu stomacal, capturées dans l'estuaire moyen du Saint-Laurent en 1979. $\overline{LS}$: longueur standard moyenne; *s* : écart-type; *N* : nombre de larves mesurées.

		Mai			Juin			Juillet		
	Station	*N*	$\overline{LS}$	(*s*)	*N*	$\overline{LS}$	(*s*)	*N*	$\overline{LS}$	(*s*)
Capelan	79−1	—	—	—	55	4,95	(0,43)	43	5,14	(0,28)
	79−2	1	4,38	—	273	4,97	(0,37)	125	4,67	(0,33)
	79−3	11	5,07	(0,34)	69	5,00	(0,38)	188	4,97	(0,38)
Éperlan	79−1	20	8,60	(0,98)	32	12,88	(1,79)	—	—	—
	79−2	—	—	—	—	—	—	—	—	—
	79−3	—	—	—	—	—	—	—	—	—
Hareng	79−1	—	—	—	2	6,31	(1,55)	34	16,40	(1,60)
	79−2	—	—	—	5	7,83	(0,24)	26	9,76	(2,42)
	79−3	—	—	—	48	8,09	(0,67)	30	11,18	(3,37)

qu'aux stations situées plus en aval ($\overline{LS} \cong 10$ mm; tableau 9). L'importance des copépodites et des copépodes adultes dans le régime alimentaire des larves de hareng en juillet est due aux grandes larves capturées à la station 79-1. Il existe en effet une relation évidente entre la taille maximale des proies et la longueur des larves de hareng. Cependant, la sélection d'une taille maximale limite peu l'utilisation de petites proies (fig. 7). Les genres *Acartia* et *Eurytemora* sont les copépodes dominants des contenus stomacaux des larves de hareng. Ces deux genres sont retrouvés en proportion équivalente.

Discussion

Coefficients et rythmes d'alimentation

Les coefficients d'alimentation sont assez faibles chez les trois espèces étudiées. Ce fait peut être expliqué en partie par le rejet du contenu stomacal des jeunes larves, consécutif à l'échantillonnage et à la fixation dans le formol (Blaxter 1965; Rosenthal et Hempel 1971; Dekhnik 1974; Colton *et al.* 1980; Hay 1981). Les valeurs que nous avons trouvées sont toutefois du même ordre de grandeur que celles citées dans la littérature. Bjorke (1976) rapporte un taux de 17 % chez les larves de capelan (4,8 à 21 mm) de la mer Barents; ce taux est similaire à ceux que nous avons observés en juillet (tableau 1). Pour l'éperlan, McCullough et Stanley (1981) mentionnent des taux variant de 4,4 à 77,8 % selon la taille des larves. Dans le cas du hareng, les taux cités varient selon la période considérée (Sherman et Honey 1971 : 50 à 60 % selon la saison; Bainbridge et Forsyth 1971 : 50 à 60 % le jour et 0 à 20 % la nuit; Cohen et Lough 1983 : 10 à 30 % en après-midi et 0 à 10 % la nuit); ces taux se comparent à ceux que nous avons observés (fig. 5c et 6b).

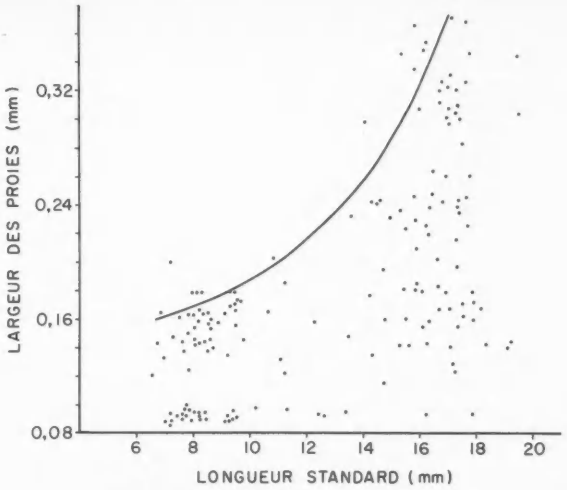

FIG. 7. Relation entre la taille (largeur maximale, appendices exclus) des proies et la longueur standard des larves de hareng. Une courbe à main levée montre l'évolution de la taille maximale des proies.

Les coefficients d'alimentation augmentent substantiellement au cours de l'été chez les trois espèces étudiées. Pour les larves de hareng et d'éperlan, cette augmentation s'explique en grande partie par l'augmentation de la taille des larves au cours de la période d'échantillonnage. Les larves de grande taille ont en effet développé une meilleure capacité natatoire (Rosenthal et Hempel 1970), ce qui leur procure une plus grande efficacité pour la capture des proies; elles sont également plus résistantes, donc moins susceptibles de régurgiter entièrement leur contenu stomacal lors de l'échantillonnage et de la fixation. Pour les larves de capelan, toutefois, aucune croissance importante n'est notée (tableau 9) à cause du transport vers l'aval des larves mises en circulation (Jacquaz *et al.* 1977; Fortier et Leggett 1982). D'un mois à l'autre, l'échantillonnage porte sur des cohortes différentes, comme on peut le déduire par la persistance des larves de capelan avec sac vitellin tout au long de la période considérée (tableau 1). Frank et Leggett (1983) et Hunter (1981) ont montré que la capacité natatoire et l'alimentation des larves dépendent de la température. En outre, les données des premiers auteurs suggèrent que le seuil optimum se situe aux environs de 8°C pour les larves de capelan. Cette température n'est atteinte qu'en juin et juillet dans l'estuaire moyen du Saint-Laurent (fig. 1). C'est également durant ces mois que les coefficients d'alimentation des larves de capelan ont été les plus élevés (tableau 1).

Courtois *et al.* (1982) et Fortier et Leggett (1982, 1983) ont décrit l'importance des phénomènes advectifs pour la distribution des larves de poisson dans l'estuaire du Saint-Laurent. La présente étude démontre que l'alimentation des larves est également influencée par la marée. La faible fréquence d'échantillonnage (3 h) et la filtration des données (moyennes mobiles), nécessaire pour l'élimination des bruits dûs à l'échantillonnage, ont cependant fait disparaître les variations de haute fréquence dans la majorité des séries représentant l'évolution temporelle du coefficient d'alimentation (fig. 4 et 5). Toutefois, grâce à l'échantillonnage plus serré (1 h) effectué à la station 79-3 en juillet, un important effet de marée a pu être mis en évidence et ce, pour les deux espèces dominantes (capelan et hareng) de notre échantillonnage (fig. 6). L'absence de corrélation avec la densité des larves présentes (tableau 7) suggère que ce cycle est davantage fonction des conditions abiotiques du milieu. Les corrélations significatives obtenues entre la température et le coefficient d'alimentation des larves de capelan et de hareng (tableau 7) suggèrent que la température, en agissant sur le rythme d'activité des larves, pourrait être responsable du cycle semi-diurne observé à la station 3. Cependant, la température ne doit pas être considérée comme le seul facteur pouvant expliquer les variations du taux d'alimentation des larves. En pareille circonstance, un gradiant décroissant serait observé de la station 1 à la station 3, ce qui n'est pas le cas. Des conditions plus favorables semblent exister pour les larves de capelan à la station 79-2, puisque leur coefficient d'alimentation y est 2 à 3 fois plus élevé qu'aux deux autres stations d'échantillonnage et ce, autant en juin qu'en juillet (tableau 1). Ceci est peut-être lié à la quantité de nourriture disponible. Il n'a toutefois pas été possible de le démontrer par notre échantillonnage, les larves de capelan se nourrissant presque exclusivement de tintinnides non retenus par le filet de 160 micromètres. Il faut donc considérer que le taux d'alimentation résulte de l'interaction entre plusieurs facteurs d'ordre physique (luminosité, température) et biologique (productivité et disponibilité de la nourriture, taille des larves).

La corrélation notée entre la densité du microplancton et le taux d'alimentation des larves de capelan n'indique pas une augmentation de ce taux en présence de proies plus abondantes lors de notre échantillonnage puisque les organismes de ce groupe sont trop gros (>100 μm) pour être ingérés par les larves de capelan (≅60 μm). Cette corrélation est davantage causée par le fait que le coefficient d'alimentation et la densité du microplancton sont tous les deux en phase avec la marée. Dans un travail antérieur (Courtois *et al.* 1982), nous avons d'ailleurs démontré l'importance des effets de marée sur la distribution du microplancton. Il n'est toutefois pas exclu que les tintinnides suivent un patron similaire.

Le principal facteur responsable des variations du coefficient d'alimentation à une station donnée demeure sans équivoque l'éclairement. Cette affirmation est appuyée par la distribution verticale des larves de capelan, d'éperlan et de hareng avec contenu stomacal. Celles-ci se retrouvent effectivement en proportion 2 à 5 fois plus grande dans la strate superficielle (0−20 m) de la colonne d'eau (tableau 5). L'éclairement semble aussi responsable de l'établissement d'un rythme circadien chez les trois espèces étudiées (fig. 4, 5 et 6). Ce rythme est d'ailleurs de plus grande amplitude que ceux liés à la marée. Ceci est visible à la station 79-3 en juillet où, tel que mentionné auparavant, l'échantillonnage a été fait à plus fine échelle (fig. 5). Ces observations confirment les résultats de nombreux auteurs concernant l'importance de la lumière pour l'alimentation des larves de hareng (Rosenthal et Hempel 1970; Bainbridge et Forsyth 1971; Blaxter 1973; Noskov *et al.* 1978) et démontrent que les larves de capelan et d'éperlan, comme plusieurs autres groupes de poissons pélagiques (saumon : Godin 1981; Terazaki et Iwata 1983; doré : Mathias et Li 1982; labridae : Duka 1976; corégone et brochet : Braum 1978) réagissent de façon similaire (« visual feeders »).

L'étude de la répartition des larves avec contenu stomacal selon la profondeur et l'éclairement porte à croire que les larves qui se sont nourries en surface le jour se laissent entraîner dans les couches plus profondes la nuit (tableau 5). Quoique moins évident, un comportement semblable est noté chez les larves sans contenu stomacal (tableau 6). La répartition des larves des trois espèces selon la profondeur et l'éclairement semble donc

le reflet d'une distribution plus homogène sur l'ensemble de la colonne d'eau durant la nuit, phénomène mis en évidence par Wood (1971) chez les larves de hareng.

Régime alimentaire

Le régime alimentaire des larves de hareng a été décrit dans plusieurs parties de son aire de distribution (Lebour 1921, 1924; Hardy 1924; Ogilvie 1927; Marshall *et al.* 1937; Bjorke 1971; Bainbridge et Forsyth 1971; Rudakova 1971; Sherman et Honey 1971; Noskov *et al.* 1978). Les résultats sont quelque peu variables selon les auteurs mais démontrent d'une façon générale l'importance des divers stades de développement des copépodes calanoïdes pour l'alimentation des larves. L'échantillonnage réalisé dans l'estuaire du Saint-Laurent en 1979 appuie cette observation, particulièrement pour les larves de hareng capturées en juillet, mais montre de plus l'utilisation de taxons moins souvent signalés (*Coscinodiscus centralis*, tintinnides, larves de cirripèdes) et met en évidence un parasitisme assez élevé particulièrement à la station d'amont (tableau 8). Il existe une relation évidente entre la taille maximale des proies et la longueur des larves de hareng, la largeur de la bouche limitant la taille des organismes que les larves peuvent ingérer (Braum 1978). Les larves de grande taille continuent cependant à ingérer de petits organismes tels des tintinnides et des œufs de copépodes, nourriture typique des larves de petite taille (fig. 7). Cette observation déjà rapportée par Sherman et Honey (1971) peut être interprétée soit comme le résultat d'une accoutumance à un type de proie donné (Rosenthal et Hempel 1970) ou comme le reflet d'un certain opportunisme, les larves capturant les proies leur fournissant le meilleur apport énergétique compte tenu des coûts associés à leur capture. Nos données ne nous permettent pas de privilégier l'une de ces hypothèses.

Les larves d'éperlan montrent un régime alimentaire beaucoup plus fortement dépendant des stades avancés de copépodes et affichent un parasitisme très inférieur à celui des larves de hareng. Les différences de régime alimentaire peuvent dépendre en bonne partie de la différence de taille observée chez les larves de ces deux espèces à une station donnée. En effet, le régime alimentaire devient semblable si l'on considère des individus de taille similaire (ex. : éperlan de la station 79-1 en juin et hareng de la même station en juillet; tableau 8).

Le régime alimentaire des larves de capelan est à toutes fins pratiques limité aux tintinnides durant toute la période d'échantillonnage (tableau 8). L'absence de changement temporel dans le régime alimentaire des larves de capelan est relié à l'absence de croissance notée précédemment.

Relations interspécifiques

Schoener (1983) a relevé de nombreux cas *in situ* de compétition interspécifique et ce, à tous les niveaux trophiques. De même, quelques travaux décrivent les effets de relations entre différentes espèces de larves de poisson (Laurence *et al.* 1981; Hunter 1976; Lett et Kohler 1976). Les résultats énoncés dans le présent travail montrent que, pour des larves de taille comparable, les diètes de l'éperlan et du hareng sont assez semblables, et il est probable que les larves de capelan utilisent des proies similaires dans certaines circonstances. Lors de son échantillonnage, Bjorke (1976) a en effet trouvé que les larves de capelan se nourrissaient principalement d'œufs et de nauplii de copépodes. De plus, les coefficients d'alimentation et les rythmes alimentaires circadiens qu'il a observés sont similaires à ceux que nous avons trouvés. Finalement, Vesin *et al.* (1981) ont également décrit l'importance des œufs d'invertébrés, des copépodes et des diatomées pour les capelans pré-juvéniles (<75 mm).

Un régime alimentaire semblable et des réactions similaires à certaines conditions environnementales ne sont toutefois pas des conditions suffisantes pour qu'il existe une compétition entre les groupes considérés. Ainsi, Courtois *et al.* (1982) ont montré que les larves de capelan, d'éperlan et de hareng, quoique présentes aux mêmes stations d'échantillonnage, se retrouvaient en fait surtout dans des masses d'eau différentes, ce qui tendait à minimiser les interactions entre ces trois groupes. Dans la présente étude, nous avons également observé que lorsque les trois espèces étaient co-occurrentes, elles étaient en grande partie de taille différente, ce qui les forçait à exploiter majoritairement des proies de taille différente. Finalement, lorsque des organismes de même taille étaient ingérés par différentes espèces de larves, ces proies appartenaient souvent à des groupes taxonomiques différents. C'est le cas notamment des grandes larves d'éperlan qui exploitent surtout les copépodes du genre *Eurytemora*, alors que les larves de hareng de même longueur ingèrent également une forte proportion de copépodes du genre *Acartia*. Govoni *et al.* (1983), dans leur étude du régime alimentaire des larves de trois espèces de poisson du golfe du Mexique, ont également montré l'absence de chevauchement des diètes.

En conclusion, nous considérons que les conditions nécessaires pour postuler qu'il existe une compétition alimentaire directe entre les larves de capelan, d'éperlan et de hareng, ne sont pas présentes dans l'estuaire moyen du Saint-Laurent.

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Different Adaptation Strategies of Atlantic Salmon (*Salmo salar*) Populations to Extreme Climates with Special Reference to some Cold Norwegian Rivers

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Jensen, A. J., and B. O. Johnsen. 1986. Different adaptation strategies of Atlantic salmon (*Salmo salar*) populations to extreme climates with special reference to some cold Norwegian rivers. Can. J. Fish. Aquat. Sci. 43: 980–984.

Some of the salmon rivers on the western and northern coasts of Norway are very cold, and the sea temperature outside these rivers is almost always higher than that in the river. Growth rates of Atlantic salmon (*Salmo salar*) parr and smolt ages and sizes have been examined in three such cold rivers. We found indications that the lower temperature limit for growth of Atlantic salmon is not a fixed temperature, but varies from population to population according to the temperature regime of their environment. Smolts are small, with average sizes of 12–13 cm total length. Females dominated in number among the smolts, but the dominance was less pronounced than in most other rivers. Strategies used by Norwegian salmon in cold rivers are therefore different from those employed by salmon in the northern extremes of the salmon's range in Canada.

Certaines des rivières à saumon qui se trouvent sur les côtes ouest et nord de la Norvège sont très froides et la température de la mer dans laquelle se jettent ces rivières est presque toujours plus élevée que celle enregistrée dans la rivière. On a étudié dans trois de ces rivières le taux de croissance des tacons de saumon de l'Atlantique (*Salmo salar*) de même que les âges et les tailles des saumoneaux. Nous avons trouvé des signes qui portent à croire que la limite inférieure de température pour la croissance du saumon de l'Atlantique n'est pas une température fixe, mais qu'elle varie d'une population à l'autre suivant le régime de température du milieu dans lequel elles vivent. Les saumoneaux sont petits, leur longueur totale étant en moyenne de 12 à 13 cm. Il y avait un plus grand nombre de femelles parmi les saumoneaux, mais cette dominance était moins prononcée que dans la plupart des autres rivières. Les stratégies adoptées par les saumons norvégiens sont par conséquent différentes de celles utilisées par les saumons dans les extrémités nord de leur aire de dispersion au Canada.

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On the west side of the Atlantic ocean the anadromous Atlantic salmon (*Salmo salar*) has its northern boundary in rivers draining into the Ungava Bay in Canada (Power 1969; Lee and Power 1976) and in the Kapitsigdlit River in Greenland (Nielsen 1961). Salmon in the Ungava rivers are exceptional in that smolts are very much larger than has been recorded elsewhere. Smolt ages are high, 4–8 yr, with a mean age of over 5 yr at migration. Smolts in the George, Whale, and Koksoak rivers averaged more than 200 mm fork length (Power 1969) and those in Leaf River attained an average fork length of 258 mm (Lee and Power 1976). There are indications that the normal life cycle is curtailed by the onset of sexual maturity and the formation of residual populations in these rivers.

The northeastern limit of salmon distribution on the European mainland is the Kara River in the western USSR (MacCrimmon and Gots 1979). The age at smolt migration increases towards the north of the salmon's range, as demonstated in Norway by Dahl (1910). In Northern Norway, Dahl (1910) and Sømme (1941) found the highest smolt ages in the extreme north, where smolts of up to 7 yr of age occurred. At a more southerly latitude in eastern Finnmark, the age at migration again decreases, and continues declining as one advances along the Murman Coast and into the southern latitudes of the White Sea (Sømme 1941; Vladimirskaya 1958; Grinjuk 1977; Jakovenko 1977).

Some rivers on the western coast of Norway are also rather cold, usually because they drain glaciers. Data on salmon growth and river temperature are available for three such rivers. In Beiarelva, the coldest of them, mean smolt age is above 5 yr, and 7-yr-old smolts are quite common. Growth of salmon parr in Beiarelva (Jensen and Johnsen 1985) is also at least as slow as that reported by Power (1973) in the Norwegian far north. There are, however, no indications that the normal life cycle is curtailed by the onset of sexual maturity and the formation of residual populations in these three rivers as shown for rivers emptying into the Ungava Bay. Growth and temperature data from these rivers may therefore give insight into the different adaptation strategies of Atlantic salmon populations to extreme climates.

Materials and Methods

Salmon parr were collected with electrical fishing gear from the Rivers Beiarelva, Saltdalselva, and Strynselva each year in late April or early May. Scale samples were taken in an area

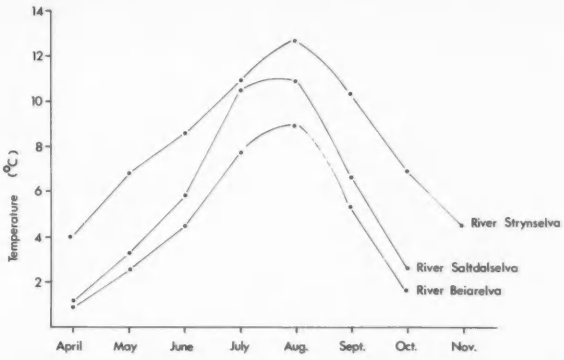

FIG. 1. Water temperature in the Rivers Strynselva, Saltdalselva, and Beiarelva. Mean data for the years 1976–83.

near the lateral line between the adipose and the dorsal fin (Dannevig and Høst 1931). Age determination was accomplished by observations of scales in water through a binocular microscope. In most cases, otoliths were also analyzed. Materials from eight different years were available from Saltdalselva, and six different years are represented in the samples from Beiarelva and Strynselva. The total number of fish analyzed from these three rivers was 586, 638, and 1396, respectively. Total fish length was measured.

Some salmon smolts were captured in the rivers each year. They were distinguished from parr by their silvery coloration and black fin edges. Only fish that were positively identified as smolts were used. A total of 108, 119, and 159 smolts were caught in Beiarelva, Saltdalselva, and Strynselva, respectively.

Temperature measurements from these three rivers, as well as from 16 other Norwegian rivers under consideration, were made by the Norwegian Water and Electricity Board, Hydrological Department.

Study Areas

The Rivers Beiarelva and Saltdalselva are situated in northern Norway (67°N, 15°E) and the River Strynselva in western Norway (62°N, 7°E). Drainage areas consist mainly of high mountains, birch forests, and in lower regions some farmland. Some of the tributaries of Beiarelva drain the glacier Svartisen, resulting in the river's grey–green color during summer. Beiarelva is also the coldest of the three rivers (Fig. 1). The two other rivers also are influenced by melt water from glaciers. Strynselva drains a large, lowland lake, Strynsvatnet (21 km^2), and therefore temperature and water flow conditions are more stable in this river than in the other two. Strynselva is situated approximately 600 km south of Beiarelva and Saltdalselva and the water temperatures in autumn and winter is considerably higher than in the two other rivers.

With the exception of the Vefsna River, Beiarelva and Saltdalselva are the largest salmon rivers in Nordland County, represented by a total annual catch of salmon, sea trout (*Salmo trutta*), and anadromous Arctic char (*Salvelinus alpinus*) of 5–8 tons. In Strynselva the total catch of salmon and sea trout is 3–5 tons annually.

Results

The mean smolt age in Beiarelva is 5.2 ± 0.2 yr (range 4–7)

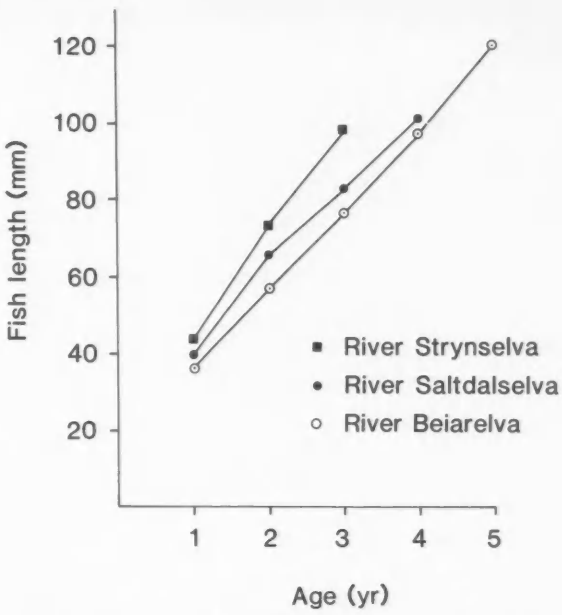

FIG. 2. Growth of Atlantic salmon parr in the Rivers Beiarelva, Saltdalselva, and Strynselva. Mean length of parr caught in April–May over 6 yr (1978–83) in the first two rivers and 4 yr (1982–85) in Strynselva.

while the corresponding values for Saltdalselva and Strynselva are 4.8 ± 0.2 (range 3–7) and 3.4 ± 0.1 (range 2–5). Mean smolt lengths in these three rivers are rather small, averaging 120–130 mm (130.2 ± 3.9 (95% CL) (n = 108), 127.0 ± 2.3 (n = 119), and 121.3 ± 2.2 (n = 159) in Beiarelva, Saltdalselva, and Strynselva, respectively). The annual growth rate (for 1+ and 2+ parr) averages 20.1, 21.0, and 27.4 mm for the three rivers, respectively (Fig. 2).

The growing season in Beiarelva (defined as the number of days with a water temperature at or above 7°C) is very short with an average length of 67 d (range 54–87). In Saltdalselva the average length of the growing season is 90 d (range 65–108), and in Strynselva it is 149 d (range 119–178). If the annual growth increment for 1+ and 2+ salmon parr is averaged into the growing season, the mean daily increment is 0.30, 0.23, and 0.17 mm for Beiarelva, Saltdalselva, and Strynselva, respectively. Figure 3 demonstrates that the correlation between growth and length of the growing season is good, and that the total number of days at or above 7°C required to produce a smolt of medium size is lowest in the coldest river and highest in the warmest one.

This trend was further examined through comparisons of data from 16 other Norwegian rivers (Fig. 4). Figure 4 illustrates that the shorter the growing season, the more effectively each day is utilized for growing. One may speculate that this is an adaptation to feeding, growth, and survival at lower temperatures in the coldest rivers. In order to test this hypothesis, the growth data from Beiarelva, Saltdalselva, and Strynselva were compared with the number of days at or above certain temperature limits by the method of least squares. At Beiarelva, best correlation was found between growth and the number of days where river temperature reached or exceeded 6.3°C (Fig. 5), while the corresponding values for Saltdalselva and Strynselva

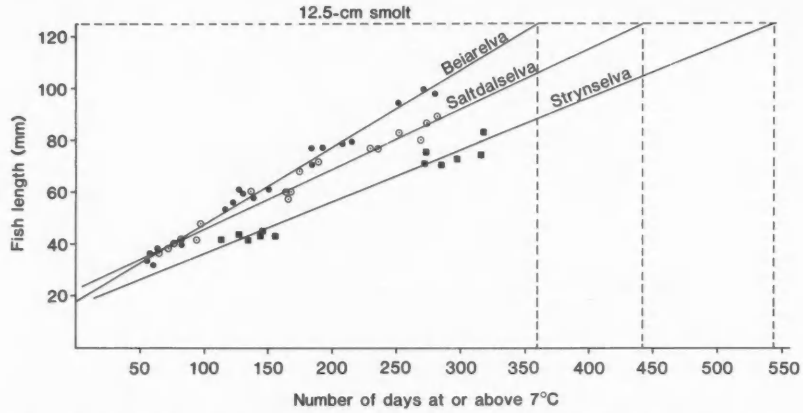

FIG. 3. Growth of Atlantic salmon parr in the Rivers Beiarelva, Saltdalselva, and Strynselva compared with the cumulative number of days with a water temperature reaching or exceeding 7°C. The growth curves are fitted to the data by the method of least squares.

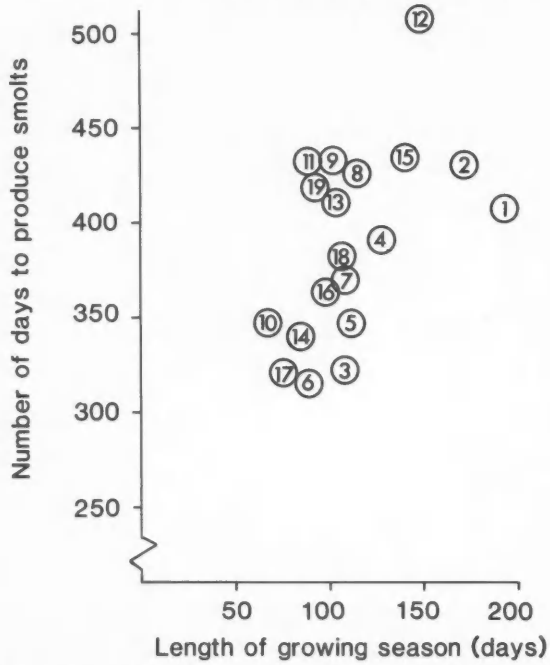

FIG. 4. Relationship between the number of days with a water temperature at or above 7°C required to produce a medium-sized Atlantic salmon smolt in some Norwegian rivers and length of the growing season (>7°C). Smolt ages for Atlantic salmon in rivers 1–9 are cited in these references: 1, River Imsa (Hansen et al. 1984); 2, Gaula i Sogn (Kålås et al. 1984); 3, Vosso (Nordland 1981); 4, Eio (Jensen and Steine 1979); 5, Orkla (Hesthagen and Garnås 1984); 6, Målselva (Andersen 1979); 7, Rauma (Arnekleiv and Koksvik 1985); 8, Driva (Korsen and Gjøvik 1977); 9, Altaelva (Heggberget et al. 1984). Nos. 10–19 are personal data from the Rivers Beiarelva, Saltdalselva, Strynselva, Hjelledøla, Jostedøla, Loelva, Vefsna, Ranaelva, Lakselva i Misvær, and Kobbelva, respectively.

were 7.3 and 7.4°C, respectively. Significant correlations were found between fish length and the number of days at or above 6.3°C ($p < 0.05$) for each of the first 3 yr of life in Beiarelva.

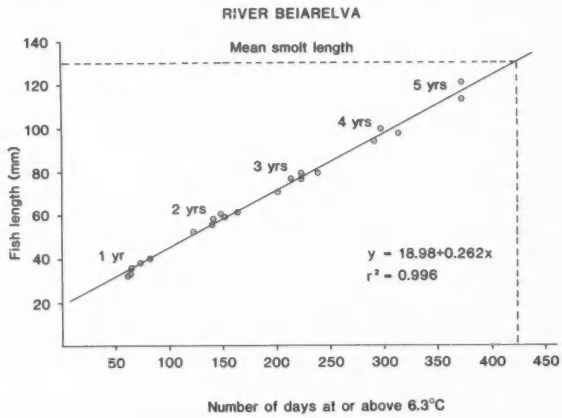

FIG. 5. Growth of Atlantic salmon parr in the River Beiarelva compared with the number of days with a water temperature at or above 6.3°C. This is the temperature threshold best fitted to growth data from this river. The material was collected in early May each year.

More females than males were found among smolts in all three rivers (Beiarelva: 44♀, 35♂; Saltdalselva: 45♀, 36♂; Strynselva: 91♀, 68♂). The sex ratio was not significantly different from 1:1 in any of them (1.26:1, 1.25:1, and 1.34:1 in Beiarelva, Saltdalselva, and Strynselva, respectively). If smolts from all three rivers are pooled, however, this is significant ($p < 0.05$).

Discussion

Allen (1940, 1941) observed that in the River Eden in England, and in the Thurso river system in Scotland, juvenile Atlantic salmon moved from riffles to pools at temperatures below 7°C, and reduced or stopped feeding. Similar observations were reported by Gardiner and Geddes (1980), who saw few young salmon in Shelligan Burn in Scotland when the water temperature fell below 5°C. Fish came out of hiding at water temperatures of 6–7°C. Based on Allen's (1969) observations that juvenile salmon growth was slow at temperatures below 7°C, Symons (1979) defined the growing season

in a river as the number of days per year in which water temperature reaches or exceeds 7°C. The same definition was used by Evans et al. (1985) while working on a river in southwestern Newfoundland.

Observations from the Matamek River in Quebec, however, suggested that Atlantic salmon parr go into hiding at temperatures somewhat higher than that previously mentioned. In the autumn, they began sheltering in rubble at 10°C, and at 9°C the majority had disappeared into hiding (Gibson 1978). Similar observations were made by Rimmer et al. (1983) on a small river in New Brunswick. Gibson's experiments indicated that parr in the Matamek River can metabolically cope with temperatures below 9°C, but that in most natural situations it may be more efficient for them to remain inactive.

In contrast, Power (1969) chose to define the growing season in the George, Whale, and Koksoak rivers in Ungava Bay as the number of days with a mean river temperature above 6°C, and in the Leaf River, also located in Ungava Bay, Lee and Power (1976) used a daily mean temperature above 5.6°C.

The River Beiarelva is an extremely cold salmon river. During two of the years investigated, the water temperature exceeded 10°C on only 8–10 d, and in 1982 the water temperature never exceeded 10°C. It is obvious that salmon in this river do not react to different river temperatures in the same manner as, for example, salmon in the Matamek River. The low temperature limit of 6.3°C for growth of Atlantic salmon in Beiarelva seems to be an adaptation to the low temperature conditions in this river, while a limit of 7.3–7.4°C found in Saltdalselva and Strynselva is probably a more normal lower temperature limit for growth in other rather cold rivers. The different lower temperature limits for growth found in these three Norwegian rivers, along with the heterogeneous observations referred to in the papers cited earlier, therefore indicate that a lower temperature limit for growth is not a fixed temperature, but varies from river to river according to the temperature conditions.

The smolt lengths in these three Norwegian rivers are among the smallest ever reported. They are, however, not smaller than can be predicted according to Elson's (1957) rule of thumb. This hypothesis states that Atlantic salmon parr transform into smolts during the season following their attaining a length of 10 cm. Although they are older, smolts at the northern extremes of the salmon's range, according to this hypothesis, are not large because of their small annual increments. Smolts of about the same size are found in some rivers in Iceland (Gudjonsson 1978), and in one river in Central Norway (Hesthagen and Garnås 1984), while in northwestern USSR the average size is somewhat longer (about 14 cm in the Porja River and 13–15 cm in the Pechora River) (Jakovenko 1977; Vladimirskaya 1958). In more southerly parts of Europe, mean smolt lengths are usually greater than 13 cm. Atlantic salmon smolts from Canadian rivers average between 13 and 18 cm in length (Dymond 1963).

An excess of females is common among samples of migrating smolts (Pentelow et al. 1933; Jones 1949; Vibert 1950; Österdahl 1969). This was also found in the Ungava rivers (Power 1969). In one of these rivers (Leaf River) the ratio was 5:1 in favor of females (Lee and Power 1976). Power (1969) explained the excess of female smolts in association with the frequent occurrence of precocious males. Precociously mature male parr remain longer in freshwater, and natural mortality thereby reduces the number of males available for migration. Precocious males have been observed in all three Norwegian rivers. More female than male smolts were also recorded, but the dominance of females seems to be less frequent than in most other rivers (Pentelow et al. 1933; Jones 1949; Vibert 1950; Österdahl 1969; Schiefer 1972; Mitans 1973; Jakovenko 1977).

The distribution of salmon in northern Canada seems to be limited by cold seawater. In rivers draining into the Ungava Bay, the daily mean water temperature in summer reaches 15°C, but the growing season is rather short. All of these rivers are large and flow northward (Leaf River northeastward) from the interior of the Labrador plateau to the coast. The climate in the basins of these rivers is considerably milder during the summer than at the outlets into Ungava Bay. The sea temperature in Ungava Bay is very low, about 0°C has been measured in late June, and approximately 2°C in August (Power 1969). The salmon has adapted to these climatic conditions by producing smolts larger than have been recorded elsewhere. They seem reluctant to leave the river, and there are indications that the normal life cycle is curtailed by the onset of sexual maturity and the formation of residual populations (Power 1969). Salmon in the Kapitsigdlit River in west Greenland have also gone a long way towards eliminating the marine phase of their life cycle, at least among a part of the population (Nielsen 1961).

Sea temperature in northwestern USSR is also low, while river temperatures there are considerably higher than in the coldest Norwegian salmon rivers. In summer, water temperature in the Pechora River, for example, may rise to 23°C (Vladimirskaya 1958), while it has never been measured higher than 16°C in the Beiarelva. Until recently, the northeastern limit of salmon distribution was the Pechora River and Varandei Island in the USSR (MacCrimmon and Gots 1979). However, apparently because of a gradual warming trend in the climate from 1919 to 1938, enhanced by a strong inflow of warm Atlantic water, salmon penetrated eastward into the Kara Sea and spawned in the Kara River from 1932 onwards (Jensen 1939; Berg 1948). In exceptional cases, specimens measuring up to 28 cm may be found in rivers in northern USSR. Large parr are particularly frequent in the Pechora River, and 5-yr-old specimens, measuring 20 cm, are not unusual in this river (Berg 1948), but the average smolt length is considerably lower than in the Ungava rivers. The overwhelming majority of fish migrate from the upper part of the Pechora River at 3 yr of age, when their average length is 13 cm. A smaller number of fish migrate at the age of 4 yr (average size 15 cm), and some at 5 yr (average size 18 cm) (Vladimirskaya 1958). In the Porja River on the Kola Peninsula, the normal smolt age is 3–4 yr, and smolt length varies from 10 to 17 cm, with an average total length of 14 cm (fork length 13 cm) (Jakovenko 1977). The same smolt age is found in the Ponoj River, also on the Kola Peninsula (Grinjuk 1977).

In cold Norwegian rivers, growth is considerably lower than in rivers both in the Ungava Bay and northern USSR. This is mainly due to low river temperatures. Sea temperature is not limiting for the distribution of salmon in this area. The sea temperature (Midttun 1975) outside Beiarelva is always higher than the river temperature, and this is usually also the case outside Saltdalselva and Strynselva. Larsson (1977) reported that if water temperature in the sea is much colder than in the river, smolts tended to linger or return to freshwater. This is probably a problem in Ungava Bay and in the northern USSR, but not in these Norwegian Rivers. One would expect, on the contrary, a selection against early smolt transformation because

of the cold river water. Small smolt size and a smolt sex ratio close to 1:1 are in accordance with this hypothesis.

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Population Structure, Ecological Segregation, and Reproduction in a Population of Arctic Char (*Salvelinus alpinus*) from Lake Tasersuaq, Greenland

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Riget, F. F., K. H. Nygaard, and B. Christensen. 1986. Population structure, ecological segregation, and reproduction in a population of Arctic char (*Salvelinus alpinus*) from Lake Tasersuaq, Greenland. Can. J. Fish. Aquat. Sci. 43: 985–992.

The population of Arctic char (*Salvelinus alpinus*) in a large lake, Tasersuaq, in West Greenland consisted of three distinct size groups representing ecological forms with differences in habitat, feeding, and reproduction. Smaller char were benthic and fed on chironomids whereas medium-sized char were living pelagically in summer, feeding on zooplankton. Larger char moved randomly over the strata, and cannibalism increased in importance with increasing size. Within each size group there was a great variation in age. All three groups contained spawners but spawning frequency variated between groups. Smaller forms probably transform to larger ones with correlated shifts in diet and periods of somatic growth only. We hypothesize that the ecological forms of the Tasersuaq population may represent an early step towards speciation. This view bridges the opposing interpretations of char populations presented in the literature considering different forms as belonging to either a common gene pool or to reproductively isolated sibling species.

La population d'ombles chevaliers (*Salvelinus alpinus*) d'un grand lac de l'ouest du Grœnland, le lac Tasersuaq, comprenait trois catégories de taille distinctes représentant des formes écologiques présentant des différences en matière d'habitat, d'alimentation et de reproduction. Les ombles de petite taille étaient benthiques et se nourrissaient de chironomidés tandis que les ombles de taille moyenne étaient pélagiques pendant l'été, se nourrissant de zooplancton. Les gros ombles chevaliers se déplaçaient au hasard au-dessus des diverses strates et le cannibalisme prenait plus d'importance à mesure que la taille augmentait. L'âge variait beaucoup à l'intérieur de chaque catégorie de taille. On retrouvait à l'intérieur des trois catégories des géniteurs, mais la fréquence de la fraye variait d'un group à l'autre. Les formes plus petites deviennent probablement plus grosses en passant par des changements correspondants du régime alimentaire et par des périodes de croissance somatique seulement. Nous émettons l'hypothèse que les formes écologiques de la population du lac Tasersuaq pourraient représenter un premier pas vers la spéciation. Ce point de vue fait le pont entre les interprétations opposées sur les populations d'omble chevalier que l'on trouve dans la littérature en considérant que les diverses formes appartiennent à un bagage héréditaire commun ou à des espèces jumelles isolées sur le plan de la reproduction.

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In recent years there has been widespread discussion on the so-called "char problem" (Nyman et al. 1981; Nordeng 1983) and on the population dynamics of arctic fish populations (Johnson 1976, 1983; Power 1978). A central issue is whether different forms of the Arctic char (*Salvelinus alpinus*) represent separate subspecies, or whether they are different ecological forms of the same species, where smaller forms may transform to larger ones. Another issue is the mechanism underlying formation of distinct size groups. These discussions have been based mainly on Scandinavian and Canadian studies.

Our objective was to describe length–frequency distribution, age composition, habitat, food segregation, and reproduction in a landlocked and unexploited population of Arctic char from Lake Tasersuaq in West Greenland and to relate the findings to the somewhat differing views on char populations presented in the literature.

Study Area

Lake Tasersuaq is situated 70 m a.s.l. at 67°10′N and 52°20′W in West Greenland (Fig. 1). The lake is 25 km long, 1–2 km wide, and has a surface area of 43 km^2. In many parts of the lake the depth is more than 200 m. It is usually ice-covered from early October to early July. Throughout the two summers of investigation, water temperatures were close to 4°C throughout the entire water column. The lake is ultraoligotrophic with a conductivity of 40 μS. The benthos is dominated by three species of chironomids (*Heterotrissocladius* spp., *Hydrobaenus* sp., and *Micropsectra* sp.) and oligochaetes. The benthos has a maximum density of 3000–4000 individuals·m^{-2} at a depth of 10 m, decreasing to about 20·m^{-2} at 200 m. The zooplankton community is practically a monoculture of the copepod *Leptodiaptomus minutus* (Liljeborg). Apart from a few sticklebacks (*Gasterosteus aculeatus*) ob-

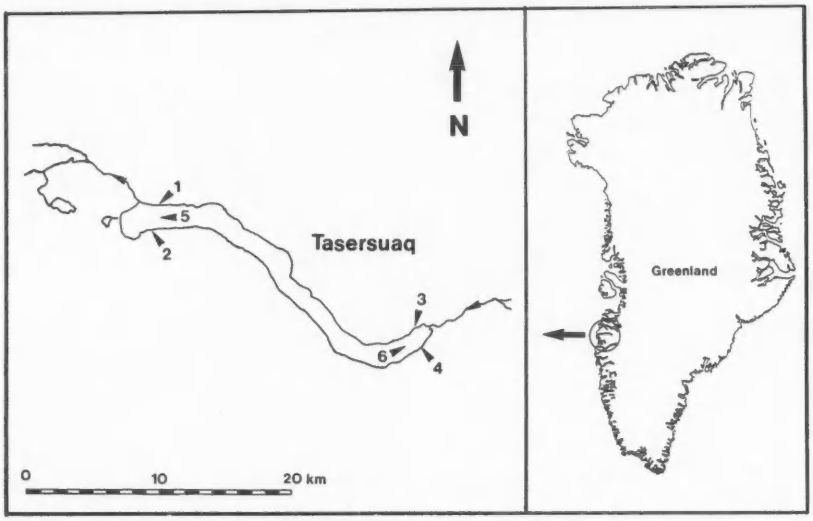

FIG. 1. Study area in West Greenland. The left map shows Lake Tasersuaq with the fishing stations: 1–4 = littoral stations; 5–6 = pelagic stations.

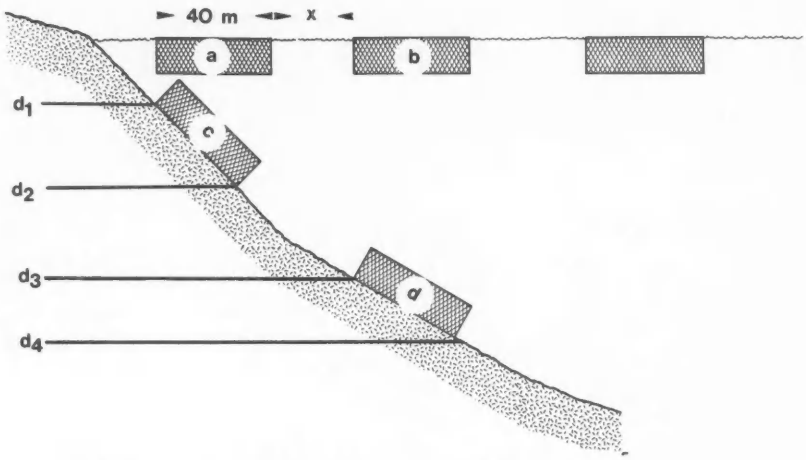

FIG. 2. Littoral station net setup with net position and depth (see Table 1).

served in one end of the lake, Arctic char is the only fish species present.

Materials and Methods

The field work was carried out in 1982 and 1983 as part of an environmental impact study. In order to sample the fish population representatively in relation to size, survey netseries were used. In 1982 the netseries were composed of eight sections (4 m wide and 1.5 m deep) with the following mesh sizes (stretched measures): 20, 25, 32, 44, 50, 60, 76, and 90 mm. In 1983, sections of 16 and 110 mm were added.

In July and August 1982, both floating and sinking nets were used near the shore to a depth of 50 m. In the pelagic zone only floating nets were used. In 1983 to facilitate statistical analysis we fished at six stations: two littoral and one pelagic in each end of the lake (Fig. 1). At every littoral station the nets were placed in four positions: two floating and two sinking nets, one of each close to the shore and the other farther out (Fig. 2; Table 1). The nets were set at night for approximately 12 h according to a scheme designed to counterbalance possible day-to-day variation in distribution between bottom

TABLE 1. Depth and position of nets at the littoral stations shown in Fig. 1 and 2.

	Depth (m)				
Station	d_1	d_2	d_3	d_4	Distance X (m)
1	1	3.5	4.5	12	20
2	1	5	7	16	20
3	1	20	20	35	0
4	1	27	27	50	0

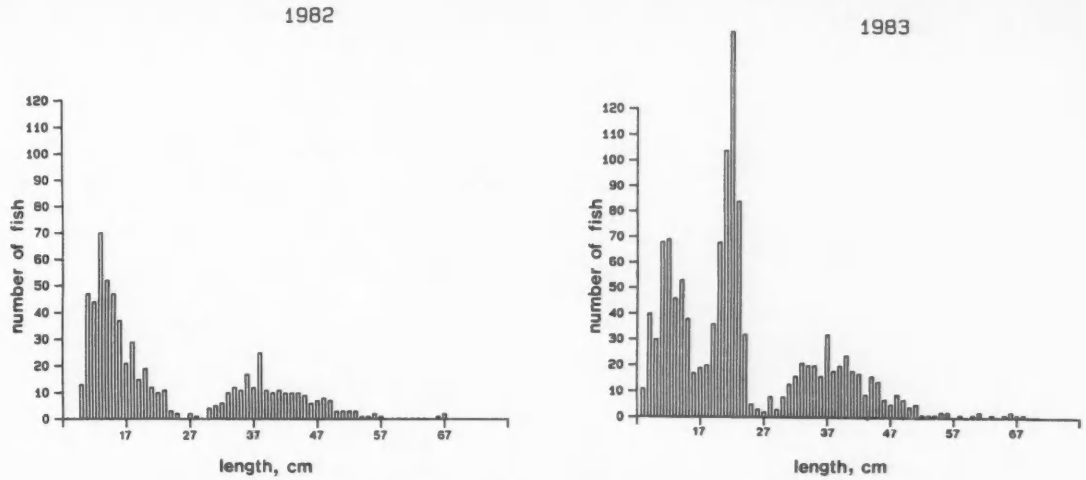

FIG. 3. Length distribution of the total catch in 1982 (July–August) and 1983 (July–September).

TABLE 2. Variance table for a reduced model of the catch containing significant effects only. Data from littoral stations.

Char size group (cm)	Effect	Degrees of freedom	p	R^2
<17	Position	3	<0.01	0.44
	Period	1	0.03	
	Residual	59		
	Total	63		
17–27	Position	3	<0.01	0.75
	Period	1	<0.01	
	Position–period interaction	3	<0.01	
	Residual	56		
	Total	63		
>27	Station	3	<0.01	0.37
	Period	1	0.01	
	Station–period interaction	3	0.04	
	Residual	56		
	Total	63		

and surface. Only floating nets were used at the pelagic stations. All stations were fished twice in the period 21 July – 4 August and twice in the period 3–13 September. To recognize possible spawning migration to the inlet river an additional net was placed in shallow water in the easternmost part of the lake and electrofishing was carried out in the inlet river during August and September. Echosounding was carried out in the pelagic zone with equipment as described by Lindem and Nunnallee (1978).

We used three-way ANOVA with first-order interactions to analyze the catch per unit effort (1 CPUE = catch in a 40-m survey net in 1 h) for the littoral stations in 1983. All effects (station, position, period, and their interactions) were regarded as deterministic, i.e.

$$X_{ijr} = \mu + a_i + b_j + c_r + ab_{ij} + ac_{ir} + bc_{jr} + E_{ijr}$$

where X_{ijr} = CPUE, μ = total mean, a_i = effects of station ($i = 1-4$), b_j = effects of position ($j = a, b, c, d$), c_r = effects of period (r = July–August, September), ab_{ij} = station–position interaction, ac_{ir} = station–period interaction, bc_{jr} = position–period interaction, and E_{ijr} = residual. The model was successively reduced to include only significant effects at the 5% significance level using the SAS program system (Ray 1982).

Fish length was measured to the nearest millimetre from the tip of the snout to the end of the tail fin as lobes were compressed. Age was determined by otolith analysis according to Nordeng (1961). In 1983 the stomach contents from 945 char were analyzed in the field using the points method of Hynes (1950) allotting 20 points to a full stomach. Food items were grouped into chironomid larvae and pupae, Trichoptera larvae and pupae, adult insects, fish, and zooplankton. Sex was determined and weight of the gonads was taken. Degree of sexual maturity was determined according to the following scale: immature ♂, testes clear and thin; immature ♀, ovary gritty but without distinct eggs; mature ♂, testes white but not swollen; mature ♀, distinct eggs with diameter <2 mm; spawning ♂, testes white and swollen; spawning ♀, egg diameter ≥2 mm.

Results

Length Distribution, Age Composition, and Habitat

In 1982 and 1983, catches were 666 and 1242 char, respectively. In 1982, the length distribution had peaks at 13 and 38 cm and only few fish of 24–32 cm were caught; in 1983, when fishing also was carried out in September, a similar picture emerged, but a prominent peak at 22 cm was added (Fig. 3).

In both years, each 5-cm length group consisted of many age groups (Fig. 4). Char in the length group with midpoint at 12 cm (e.g.) were from 2 to 16 and 3 to 13 yr old in the two years, respectively. The prominent peak found in 1983 at 22 cm consisted of char from 5 to 13 yr. The larger char (>30 cm) had ages ranging from 6 to 24 yr.

The length distribution indicated that three length groups should be distinguished for analysis of the material: (1) char smaller than 17 cm, (2) char from 17 to 27 cm, and (3) char

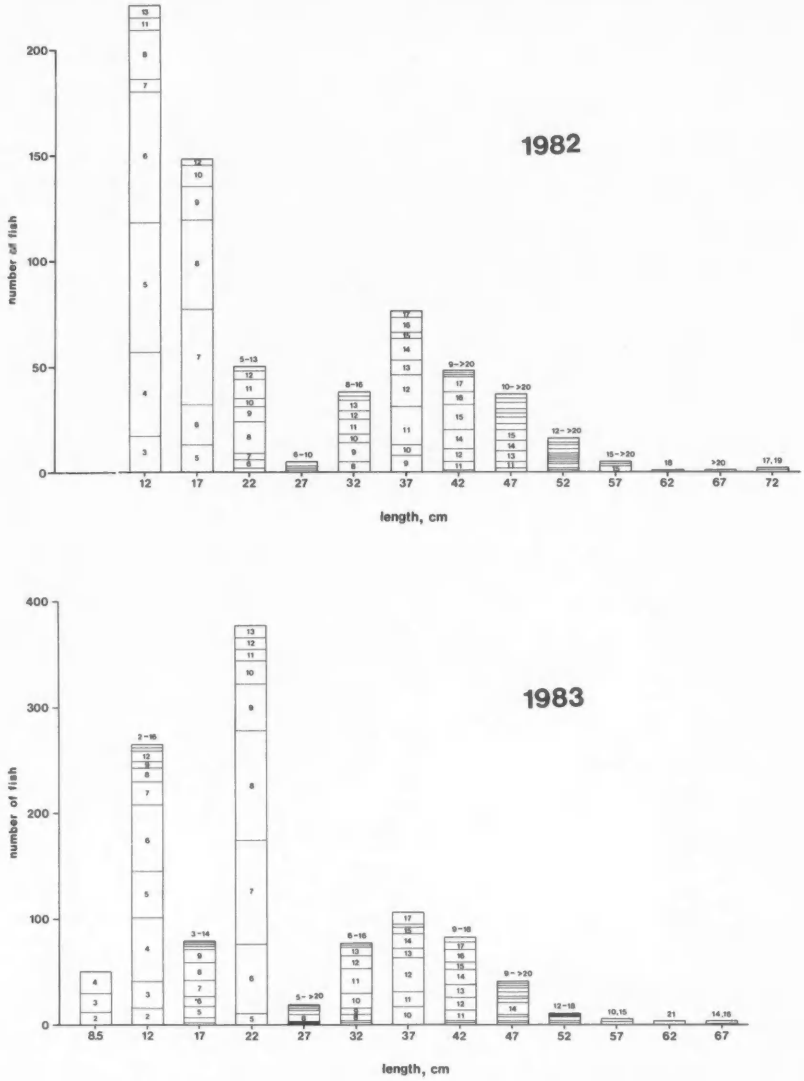

FIG. 4. Contribution of age classes in 5-cm length groups in 1982 and 1983.

larger than 27 cm. The CPUE in 1983 was analyzed for the three length groups.

For the length group less than 17 cm, CPUE was mainly affected by position and period as indicated by the variance table for a reduced model containing significant effects only (Table 2). The model explained 44% of the total variation. The CPUE was much greater in the sinking nets than in the floating nets (Table 3). There were no significant differences between the two sinking nets (positions c and d in Fig. 2) or between the two floating nets (positions a and b) (Duncan test).

Position, period, and position–period interactions had significant effects on the CPUE for char 17–27 cm (Table 2). The model explained 75% of the total variation. Position–period interaction implies that the position had different impact on CPUE in the two periods. There was a great increase in CPUE from July–August to September for the two sinking nets and the floating net near the shore (position a), whereas the increase in CPUE was inconsiderable for the floating net farthest from the shore (Table 3).

For char greater than 27 cm, station, period, and station–period interaction were significant (Table 2). The model explained 37% of the total variation. The mean CPUE for the different stations and in the two periods is seen in Table 3. The CPUE was generally largest for stations 3 and 4 and in July–August. While CPUE at station 1, 3, and 4 decreased from July–August to September, CPUE at station 2 increased, giving the significant station–period interaction.

Feeding

The different length groups of char showed different food preference (Fig. 5). For char less than 17 cm, the dominant food items in both periods were chironomid larvae and pupae,

TABLE 3. Catch per unit effort (CPUE) for the three size groups. For char <27 cm the littoral stations are pooled, as they did not show significant differences; similarly, net positions are pooled for char >27 cm. 1 CPUE is the number of fish caught during 1 h in a 40-m net series.

Char size group (cm)	Period	Position (littoral stations 1–4) a	b	c	d	Pelagic stations
<17	July–August	0.06	0.01	0.55	0.44	0.00
	September	0.21	0.00	0.93	0.78	0.00
17–27	July–August	0.01	0.03	0.09	0.08	0.06
	September	0.82	0.11	1.59	0.67	0.06
		Littoral station (positions a–d) 1	2	3	4	
>27	July–August	0.22	0.12	0.56	0.33	0.13
	September	0.09	0.19	0.23	0.24	0.08

pupae being most important in July–August and larvae in September. Trichoptera larvae were taken to some extent by the smallest fish in this group, and zooplankton by the largest. Char of 17–27 cm fed mainly on chironomids in the first period, especially pupae and imagines, while zooplankton was predominant later (September). In September, nearly all char of 22 cm, which were ready to spawn and contained food, had only zooplankton in the stomach. For char larger than 27 cm, chironomid larvae and pupae and adult insects were less important; cannibalism was increasingly important with increasing fish length. Trichoptera were also important for this length group. Of all the spawning char 73% had empty stomachs, while for nonspawners the percentage was only 28.

Food selection was more pronounced in September than in July–August. In September, 77% of the char containing food had only one kind in the stomach, main items being chironomid larvae, Trichoptera larvae, zooplankton, or char. In July–August only 26% of the stomachs contained a single item.

Reproduction

Direct observations of spawning grounds were not made, and there was no difference between the frequency of spawners caught at the different littoral stations. The additional net near the inlet did not catch particularly many spawners and no spawners were caught by electrofishing in the river.

Significantly more males than females were caught (Table 4). The difference in sex ratio was most pronounced among the smaller char, and especially for the medium-sized char (17–27 cm) in 1983. The males were the first to arrive at the spawning grounds near the shore. This was indicated by the fishing at the stations in the west end of the lake in September 1983 where sampling was carried out over a 9-d interval. Among the ripe char at these stations the sex ratio was 100:21 on 3–4 September (binomial test $\delta : ♀ = 1:1$, $p < 0.01$) and 55:44 on 12–13 September ($p > 0.15$).

The frequency of spawning increased from 6% at a length of 8 cm to 97% at 22 cm for the char caught in 1983. After this there was an abrupt drop in frequency to a minimum of 8% at 28–30 cm and then again there was a rise with increasing size (Fig. 6). The youngest spawning char in the length groups

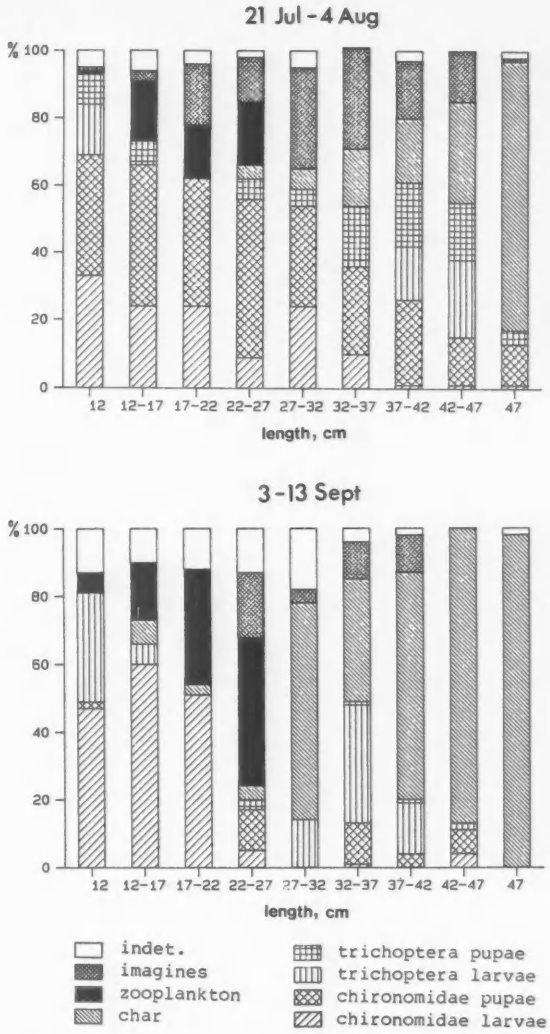

FIG. 5. Food preference. Composition of food items (percent) for 5-cm length groups in July–August and September 1983.

less than 17 cm and 17–27 cm were 3 and 5 yr, respectively. Many char were sexually mature in their fourth, fifth, or sixth year of life, but immature or nonspawning older char were common. Most of the char less than 27 cm in the age groups 7, 8, and 9 were sexually mature, while in the same age groups all of the larger char were immature or nonspawning (Fig. 7). The youngest larger spawning char was 10 yr old.

Just prior to the spawning period (September 1983) the gonads constituted a mean of 3.3% (max. 4.3%) for males and 9.2% (max. 16.1%) for females of the total wet weight.

Discussion

For the years of investigation a bi- or trimodal length distribution was observed. Analysis of the CPUE data from 1983 shows that the influence of position, period, and station on the catch varies considerably between the length groups (Tables 2

TABLE 4. Sex distribution of char from the three length groups. A 1:1 distribution was tested with the binomial test.

	Char size group (cm)							
	1982				1983			
	<17	17–27	>27	Total	<17	17–27	>27	Total
Number of ♂	182	67	126	375	205	312	172	689
Number of ♀	132	43	105	280	177	197	172	546
p	<0.05	<0.05	ns	<0.001	ns	<0.001	ns	<0.001

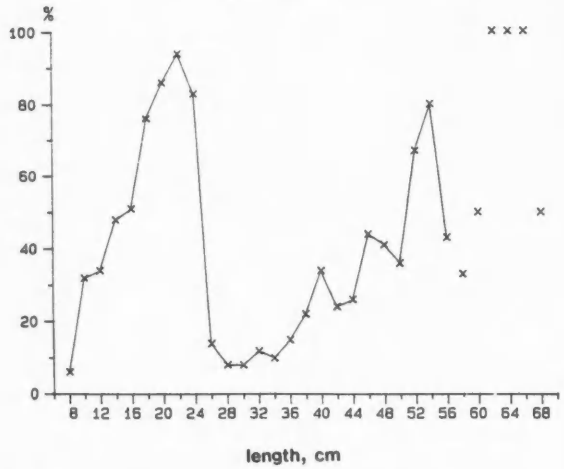

FIG. 6. Frequency of spawners for different length groups. Groups with less than five fish are not connected.

and 3). Net selectivity also makes it difficult to compare the catch of different length groups and to estimate the true composition of the total population. It seems though that there are distinct peaks in the length distribution of the population, and a similar pattern has been observed in many Canadian populations of char, lake trout (*Salvelinus namaycush*), and lake whitefish (*Coregonus clupeaformis*) (Johnson 1976) as well as in other unexploited populations of Arctic char in Greenland (Sparholt 1985). The difference between the catches in 1982 and 1983 is attributed to the different fishing periods; in 1983, pelagic fish moving to the shore for spawning were added.

To explain such length distributions, Johnson (1976, 1983) suggested a model whereby some intraspecific feedback mechanism controls the growth of small-sized "juvenile" char into a group of large "established" char. In Lake Tasersuaq the fish within the very narrow peak at 22 cm show the characteristics of an "established" group viz. little variation in length and large variation in age. The peak around 37 cm is composed of old char. This wider peak is probably the result of a piling-up process of old slow-growing char as suggested by Power (1978), but net selectivity may also be a factor, larger fish being caught more efficiently, partly due to a greater mobility of predatory fish. The fact that very few fish in the length interval 25–30 cm were caught can be explained by a period of rapid growth for char of this size.

This interpretation implies that individual fish are able to transform from one group to another. We have not been successful in demonstrating such shifts in the population in Tasersuaq by reading otoliths, but the age and length composition described indicates that such transformations do take place. Similar shifts have been shown by Nordeng (1983) through rearing and transplantation experiments in the Salangen River system in Norway, where "small resident" can transform to "large resident" and even further to anadromous char. Based on otolith readings, Skreslet (1973) also concluded that char from Lake Nordlaguna in Jan Mayen are able to shift between periods with production of sexual products and periods of somatic growth only.

Segregation of the different size groups by habitat is shown by the analysis of catch data. Char less than 17 cm mainly live close to the bottom down to at least 50 m. Medium-sized char of 17–27 cm lead a pelagic life in summer (July–August), but come close to shore in September to spawn. The reason for the low numbers caught at the pelagic stations is probably low densities in the pelagic zone and a main distribution below the upper water layers fished. Echosounding transects of the lake showed char widely dispersed in depths from 25 to 80 m. Char larger than 27 cm apparently move around over all the strata fished.

Ecological segregation of the three size groups is further indicated by the analysis of stomach contents (Fig. 5). While the small char mainly feed on chironomids, the pelagic char (17–27 cm) feed on zooplankton and the larger char are partly cannibalistic. Cannibalistic char have been reported from other arctic lakes where char is the only fish species present, e.g. in Jan Mayen (Skreslet 1973), Svalbard (Gullestad 1975), and Greenland (Sparholt 1985). Food segregation was most pronounced in late summer as was found by Nilsson and Filipsson (1971) and Sparholt (1985).

All three length groups included spawning and nonspawning char, but the frequency of spawners varied with fish size (Fig. 6). Some char were observed to be sexually mature when only 8 cm long or 3 yr old, while many large old char had unripe gonads (Fig. 7). Whereas it seems that char less than 27 cm in the age groups 7–9 have a yearly spawning cycle, a few large fish with otherwise empty ovaries were found to contain a few large eggs. This is considered an indication that spawning took place in previous years and a spawning cycle of more than 1 yr seems to be the rule for the larger fish in the population. A spawning cycle of more than 1 yr was also recorded by Skreslet (1973), Gullestad (1975), and Sparholt (1985). For char above 28 cm, spawning frequency increased with increasing fish length (Fig. 6). This could be interpreted as late sexual maturation but more likely seems to be a "rematuration" as found by Skreslet (1973) and Nordeng (1983).

The literature presents two alternative interpretations of char populations. One concept considers that several reproductively isolated "sibling species" occur, environmental factors determining their distribution and abundance (Nilsson and Filipsson

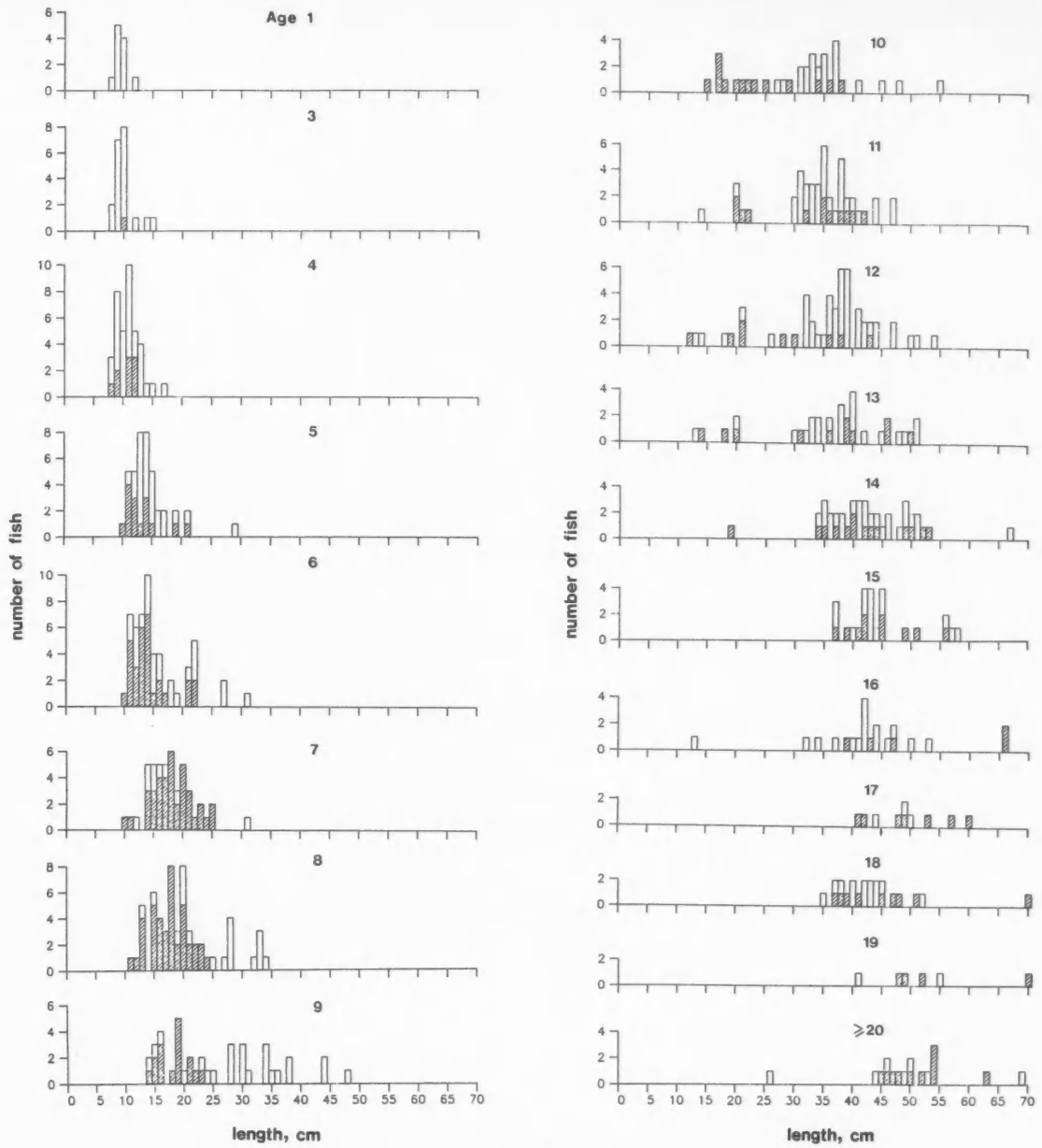

FIG. 7. Length distribution of each age class of spawning char (hatched) and nonspawners (open).

1971; Nyman 1972; Klemetsen and Grotnes 1980; Nyman et al. 1981). This is not supported by our findings. The char population in Tasersuaq is divided into three rather separate groups, but there is evidence of transformation between groups. Furthermore, analysis of serum esterase allel combination for 101 char larger than 17 cm by J. Hammar (Institute of Freshwater Research, S-17011 Drottningholm, Sweden, pers. comm.), according to the method described by Nyman (1967), did not indicate that more than one population of char was present in Tasersuaq.

According to the alternative "conspecific" concept the char is an ecologically polymorph species able to adapt to different ecological niches (Savvaitova 1980), different morphs developing from a common gene pool (Nordeng 1983). The number of char morphs may depend on the number of niches available as suggested by Hindar and Jonsson (1982). This is in good accordance with the occurrence of three forms in the large deep lake Tasersuaq where, apart from a few sticklebacks, Arctic char is the only fish species present. The large differences in ecology and reproduction between the size groups indicate,

however, that the morphs may become more or less isolated.

Seen in an evolutionary context the two concepts can be bridged by assuming a divergent development from a common gene pool to reproductive isolation. The driving forces in the model of Johnson (1976) can sustain a stable state in the population, but also be a major factor in this process. Homing to spawning grounds and imprinting could, as concluded by Frost (1965), isolate the char morphs, and given time and steady conditions produce reproductively isolated populations. The ecological forms of the Tasersuaq population may represent an early step towards sympatric speciation.

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Long-Term Dynamics of Three Crayfish Species in Trout Lake, Wisconsin

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Previous reports suggested that crayfish species composition in northern Wisconsin is changing unidirectionally because of the competitive hierarchy of sequential invaders. In Trout Lake, Vilas County, Wisconsin, we expected *Orconectes virilis* to decline in the presence of *O. propinquus* and for both to be displaced by *O. rusticus*. Our data show the abundance and species composition of *Orconectes* species in Trout Lake have been changing since 1931. A 1932 survey determined that *O. virilis* was the only *Orconectes* in Trout Lake. In 1973, we found that *O. propinquus* was abundant and *O. virilis* uncommon. Yet between 1973 and 1983 (probably between 1980 and 1983), *O. virilis* became much more widespread and abundant, while the population of *O. propinquus* declined. Between 1973 and 1979, *O. rusticus* invaded the lake, but has remained in low abundance. Both of our predictions are contradicted by our results. *Orconectes propinquus* has declined and *O. virilis* has increased, and while *O. rusticus* has invaded, it has not yet displaced the other species. We suggest that the outcome of interspecific interactions is variable and probably affected by other community structuring forces such as predation, parasitism, and disturbance, in addition to competition.

Des rapports précédents donnent à penser que le changement des espèces d'écrevisses au nord du Wisconsin s'est fait dans une seule direction en raison de la hiérarchie au point de vue de la concurrence des envahisseurs qui se sont succédés. Au lac Trout, comté de Vilas, au Wisconsin, nous nous attendions à ce que *Orconectes virilis* régresse en présence d'*O. propinquus* et que ces deux espèces soient supplantées par *O. rusticus*. Nos données montrent que l'abondance et la composition des espèces d'*Orconectes* dans le lac Trout ont changé depuis 1931. Une étude réalisée en 1932 a permis d'établir que *O. virilis* était la seule espèce d'*Orconectes* présente dans le lac Trout. En 1973, nous avons trouvé qu'*O. propinquus* était abondant et qu'*O. virilis* était rare. Cependant, entre 1973 et 1983 (probablement entre 1980 et 1983), *O. virilis* est devenu plus répandu et abondant, tandis que la population d'*O. propinquus* a diminué. Entre 1973 et 1979, *O. rusticus* a envahi le lac mais son nombre est demeuré faible. Nos deux prédictions sont contredites par les résultats que nous avons obtenus. *Orconectes propinquus* a diminué et *O. virilis* a augmenté en nombre, et, bien que l'espèce *O. rusticus* ait envahi le lac, elle n'a pas encore supplanté les autres espèces. Nous pensons que le résultat des interactions interspécifiques est variable et qu'il est probablement influencé par d'autres forces qui modèlent la communauté comme la prédation, le parasitisme et le dérangement en plus de la concurrence.

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Observations of plants and animals expanding their ranges have provided many insights into the roles of environmental conditions, predators, and competitors in determining the distributions of organisms (Elton 1958; DeBach 1974; Parsons 1983; Simberloff 1981). We present data from Trout Lake in north-central Wisconsin on the long-term dynamics of a crayfish assemblage characterized by sequential species invasion. Previous investigators of crayfish species assemblages have suggested that interspecific competition was the most important factor in determining local distribution among *Orconectes* species in northern Wisconsin lakes and elsewhere (Bovbjerg 1970; Rorer and Capelli 1978; Flynn and Hobbs 1984; cf. Butler and Stein 1985). In aquarium experiments offering limited shelter to equal-sized adult individuals of three *Orconectes* species, there was a clear hierarchy of aggression and shelter occupancy favoring *O. propinquus* over *O. virilis*, and *O. rusticus* over both other species (Capelli and Munjal 1982). In other experiments, juvenile *O. rusticus* likewise dominated equal-sized juveniles of the other species (P. A. Medvic and J. J. Magnuson, Center for Limnology, University of Wisconsin-Madison, Madison, WI 53706, unpubl. data). There have been sequential species invasions and replacements in some lakes (Capelli 1982).

The only common crayfish in the lakes of northern Wisconsin in the early part of this century was *O. virilis* (Hagen) (Creaser 1932). Two congenors, *O. propinquus* and *O. rusticus*, have since expanded their ranges from Illinois

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and other more southerly areas to include many lakes in northern Wisconsin (Capelli and Magnuson 1983) and eastern Ontario (Crocker and Barr 1968; Berrill 1978). *Orconectes rusticus* now also occurs in lakes on the Ontario–Minnesota border (S. Serns, Wisconsin Department of Natural Resources, Northeast Area Headquarters, Woodruff, WI 54568, pers. comm.). *Orconectes propinquus* (Girard) first invaded the Lake Michigan drainage of eastern Wisconsin before moving into the lakes of the Mississippi and Lake Superior drainage of northern Wisconsin (Creaser 1932; Capelli and Magnuson 1983). *Orconectes propinquus* is now common in northern Wisconsin, apparently having replaced *O. virilis* in many lakes. In the last 30 yr, *O. rusticus* (Girard) has spread rapidly, probably with human help (Capelli and Magnuson 1983). In many northern Wisconsin lakes, *O. rusticus* has invaded and apparently displaced both *O. virilis* and *O. propinquus*, but with different rates of increase in different lakes (Capelli 1982). In 5 yr, the proportion of *O. rusticus* in the total crayfish population changed little in some lakes, but in one of nine lakes studied went from 11 to 100% of the total population (Capelli 1982).

In northern Wisconsin lakes, any impact associated with crayfish invasions has a relatively low frequency (two crayfish species invasions have occurred in the last 50 yr), probably a long duration (years or decades), and a potentially large magnitude. *Orconectes rusticus* achieves extremely high population densities (Lorman 1980), eats lake trout (*Salvelinus namaycush*) eggs (Horns and Magnuson 1981), and has been implicated in the recruitment failure of walleye (*Stizostedion vitreum*) and other sport fishes (A. Ensign, Wisconsin Department of Natural Resources, Rhinelander, WI 54501, pers. comm.). In both natural and experimental situations, it has been linked to dramatic declines in macrophytes (Magnuson et al. 1975; Lorman and Magnuson 1978; D. M. Lodge and J. G. Lorman, unpubl. data). Whether *O. virilis* or *O. propinquus* significantly altered the communities they invaded is not known.

In no single lake have crayfish populations been monitored long enough to discover whether observed invasions inevitably involve permanent species displacements and changes in the community. The competitive relationships among the three *Orconectes* congenors may change over time, perhaps mediated or reversed by adaptations or shifts in species composition of prey plants and invertebrates or by adaptations of predators and competitors to the invader's presence. Here we use data spanning more than 10 yr to document concurrent changes in the abundance of *O. propinquus*, *O. virilis*, and *O. rusticus* in Trout Lake, Wisconsin.

Study Site

Trout Lake, one of the largest (1548 ha; 30-km shoreline) in north-central Wisconsin (Vilas County, 46°N, 89°W), is a mesotrophic lake (pH = 8.0; specific conductance = 85 μS/cm) with calcium levels (11 mg/L) well above those which may limit crayfish (unpublished physico-chemical data from Northern Lakes LTER Project, J. J. Magnuson, Center for Limnology, University of Wisconsin-Madison; Capelli and Magnuson 1983). Because traps were set inshore, and crayfish abundance is related to substrate (Lorman 1980), we visually surveyed littoral substrates. Most of the shoreline is a mixture of cobble and sand, with only a few vegetated areas. Along the eastern shore of the south basin, the substrate is dense cobble. Small rosulate macrophytes (e.g. *Eleocharis*, *Isoetes*, and *Lobelia*) occur on moderately protected sandy bottoms. Larger macrophytes (e.g. *Vallisneria*, *Myriophyllum*, *Bidens*, *Ceratophyllum*, and *Potamogeton*), which have been affected by *O. rusticus* in other lakes, occur on finer organic sediments, especially in three areas of the lake: the small bay pinched from the western shore of the north basin, parts of the southwestern corner of the south basin, and the bay in the southeastern corner of the south basin (Wilson 1941).

TABLE 1. Description, by shoreline segment (see Fig. 1), of crayfish sampling effort, 1970–84.

Segment	Date	No. of traps
A	Summer 1970	~100
	1 Aug. – 27 Sept. 1973[a]	14
	22–23 Aug. 1983[a]	10
B	1973	22
	1983	8
C	Summers 1971 and 1972	>100
	1973	54
	10 Aug. 1979	8
	20 Aug. 1980	10
	11 Aug. 1981	5
	1983	3
	16 Aug. 1984	21
D	1973	20
	1983	6
E	1973	7
	1983	5
F	1973	5
	1983	6
G	1973	7
	1983	7
H	1973	8
	1983	6
I	1973	4
	1983	4
J	1973	3
	11 Aug. 1981	5
	1983	7
K	1973	2
	1983	2
L	1973	7
	11 Aug. 1981	10
	1983	7
M	1983	8

[a]All succeeding 1973 and 1983 dates refer to these days.

Materials and Methods

Crayfish were sampled with wire-mesh cylindrical (21-cm diameter) funnel-ended minnow traps during summers of the years 1970–73, 1979–81, and 1983–84. Trap openings were widened to 3.5–4.5 cm diameter. Capelli and Magnuson (1983) found that, in terms of relative species abundance, results of direct censuses by divers using SCUBA were strongly correlated with trapping results.

Traps usually catch more male than female *Orconectes* (Brown and Brewis 1978; Malley and Reynolds 1979; Capelli 1982), with female and male catches changing independently with time as a function of sex-dependent life cycle phenomena. Therefore, we report data only for males, using number of males as an index of the total population (hereafter referred to as "trap index of abundance").

In 1973 and 1983, the entire shoreline was sampled. In other years, only some segments of shoreline in the south basin were sampled (Table 1). In 1973, 1979, 1980, 1983, and 1984, traps were baited with 120 g of beef liver and separated by >40 m or by a time interval >2 d. In other years, traps were baited with variable amounts of liver and were separated by smaller distances (>5 m); results for those years are, therefore, not included in comparisons of absolute crayfish abundance, but are used in comparisons of relative abundance. Traps were always placed at depths of 1–3 m, and checked 22–26 h later.

Traps were set singly at various times during the day, from midmorning to late afternoon. A trapping experiment conducted concurrently with the 1984 trapping season showed that time of deployment had no effect on total catch of traps set for 24 h. Traps set at 09:00 in randomly assigned sites on the eastern shore of Trout Lake caught the same number of male crayfish ($\bar{x} \pm SE = 9.7 \pm 1.5$, $n = 7$) as traps set at 15:00 (10.3 ± 1.9, $n = 7$, $t = 0.24$, not significant).

Except for the summer long trapping sessions of 1970, 1971, and 1972 (from which we use data only for relative species composition), trapping occurred only during intermolt periods (see Capelli 1975; Stein 1977): late May – early June (when males are in Form I, the sexually competent form), mid-July (males in Form II), or mid-August–September (males in Form I). Water temperature always exceeded 13°C, the point above which catches are insensitive to temperature (Capelli 1975). We thereby avoided seasonal changes in trapability.

Abundance data for all species combined were compared for 1973 and 1983 using *t*-tests based on unequal sample sizes. Species composition for 1973 and 1983 was compared with the *t*-statistic based on arcsine $\sqrt{p}$ transformation of *O. propinquus* data (Sokal and Rohlf 1969, p. 608).

Voucher specimens from 1971 and 1983 have been deposited in the University of Wisconsin Museum of Zoology.

Results

Both the trap index of abundance of crayfish and the relative abundance of component species changed between 1973 and 1983 (Fig. 1). The trap index of abundance of crayfish (all species pooled) declined, with significant declines of about 50% (from 25–40 per trap to 5–15 per trap) occurring along the southeast and east shores of the south basin (Fig. 1A). Concurrent with the decline in total abundance was a shift in composition from *O. propinquus* to *O. virilis* (and *O. rusticus*) (*t*-test, $p < 0.05$; Fig. 1B), with significant shifts largely coinciding with significant declines in total abundance (Fig. 1).

In 1973, *O. propinquus* was by far the most widely distributed and abundant crayfish within Trout Lake. Total trap catches were high (25–40 per trap in the eastern half of both basins), and *O. propinquus* comprised 80–100% of the catch in most parts of the lake. *Orconectes virilis* was the only other species present. Along the eastern shores of both basins, *O. virilis* comprised only 1% of the total crayfish population. In most other parts of the lake, *O. virilis* comprised 3–38% of the total. *Orconectes virilis* was more abundant than *O. propinquus* in only two shoreline segments, the northeast (Segment F, 55%) and the southwest (Segment I, 67%). Earlier data (for which catch effort was not recorded) were consistent with the lakewide patterns of 1973. *Orconectes propinquus* comprised >99% of 3300 crayfish trapped in Segment A during 1970 and 100% of several thousand crayfish from Segment C in 1971–73.

Between 1973 and 1983, the relative abundance of *O. virilis* increased dramatically, reflecting a small but significant increase in the trap index of abundance. For example, along the east shore (Segments A–D), *O. virilis* increased from <1% (of total catches of 25–40 per trap) to 25–50% (of total catches of 5–15 per trap).

Orconectes rusticus invaded Trout Lake between 1973 and 1979. By 1983 it comprised 2–16% of the trap catches in only two (B and C) of 13 shoreline segments. It has remained limited in numbers and distribution.

Data from other years (available for shoreline Segment C only) provided better time resolution for the changes in abundance for each crayfish species (Fig. 2). Along shoreline Segment C, trap catches of *O. propinquus* declined from about 30–40 per trap in 1973, 1979, and 1980 to about 10 per trap in 1983 and 1984. Trap catches of *O. virilis* exhibited an opposite pattern. *Orconectes virilis* did not occur in Segment C in 1973, 1979, and 1980, but by 1983, catches were 4 per trap. *Orconectes rusticus* was first caught in Segment C in 1979, and has remained in low abundance. In summary, the trap index of abundance of *O. propinquus* in Trout Lake declined dramatically between 1973 and 1983. During the same period, the trap index of abundances of *O. virilis* and *O. rusticus* increased. Consequently, the lakewide relative abundance of *O. virilis* increased dramatically. The relative abundance of *O. rusticus* increased in only a small part of the lake.

Discussion

Because we avoided many trapability problems by using a standard amount of bait, reporting only male catches, and trapping only during intermolt periods, our sampling detected real changes in crayfish abundance and species composition. We suggest that factors in addition to interspecific competition are important in determining the absolute and relative abundances of crayfish species. As evidenced by our 10-yr records, the abundances and species composition of crayfish in Trout Lake are dynamic, characterized by species invasions, partial species replacements, and probably also by species population rebounds.

Explanations for the decline in abundance of *O. propinquus* between 1973 and 1983 and concurrent increase in abundance of *O. virilis* and *O. rusticus* must be based on differences in biology or history of the populations. In most respects, however, the species are apparently similar. Each prefers cobble substrates (Capelli 1975; Berrill 1978; Lorman 1980) and has an opportunistic omnivorous diet (Lorman 1975; Capelli 1980). In addition, our observations and those of Berrill (1978) suggest that the life cycles of the three *Orconectes* species are synchronous. However, growth rates may not be the same, and mean adult size is not the same. *Orconectes rusticus* and *O. virilis* achieve similar carapace lengths, but the chelae of *O. rusticus* are larger. Both *O. rusticus* and *O. virilis* are considerably larger than *O. propinquus* (Capelli 1975). Since size is an important determinant of success in aggressive interactions, the competition model is inadequate in ignoring the

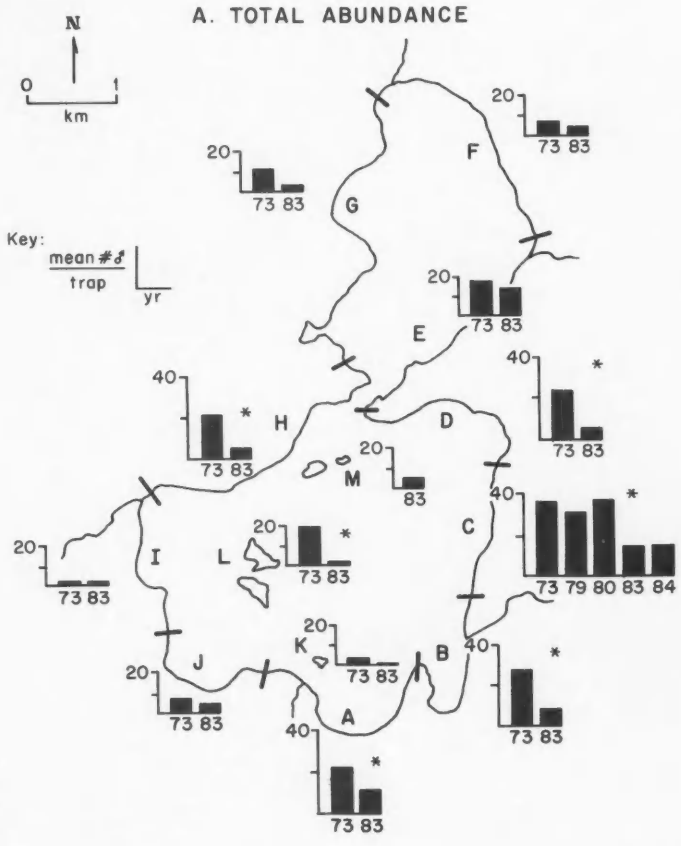
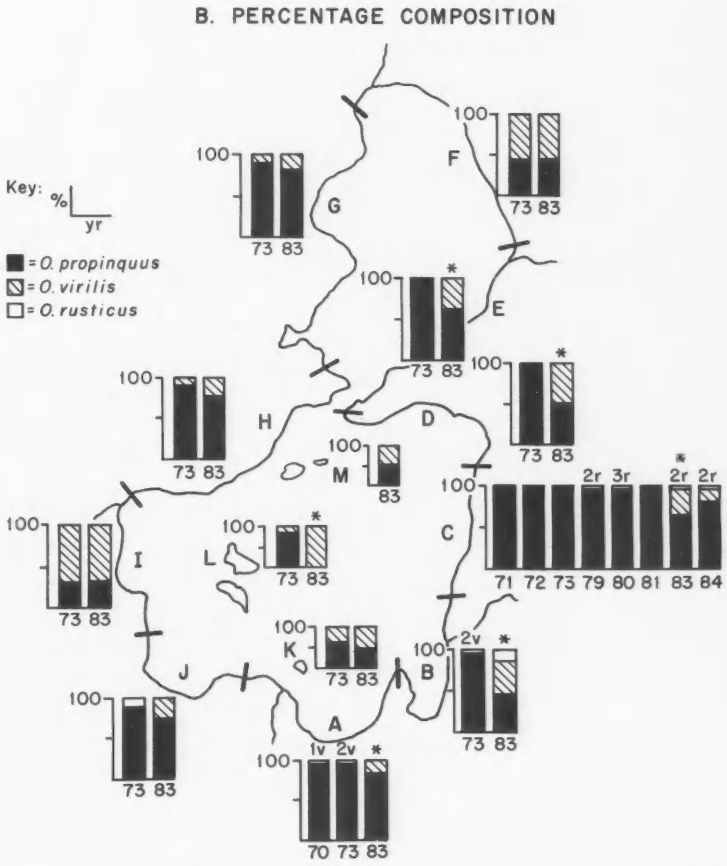


FIG. 1. (A) Trap index abundance of all species combined and (B) percentage species compositi
Trout Lake during the period 1970–84. Significant differences (t – test, $P < 0.05$) in total
with an asterisk. In Fig. 1B, very small percentages are given numerically above the histogram
for Segment M.



omposition of *Orconectes* male crayfishes for shoreline segments (designated with capital letters) in in total crayfish abundance and in percentage species composition between 1973 and 1983 are noted istogram, e.g. "2r" denotes 2% *O. rusticus*, "2v" denotes 2% *O. virilis*. No 1973 data are available

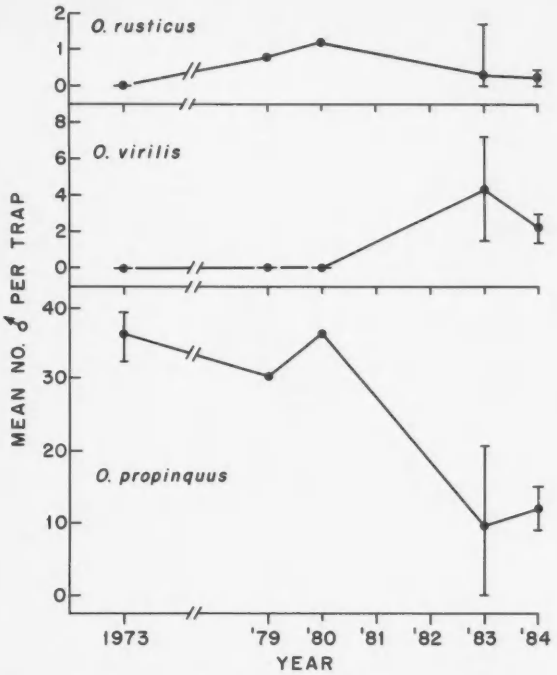


FIG. 2. Trap index of abundances ($\bar{x} \pm 95\%$ confidence interval) of *Orconectes* male crayfishes at shoreline Segment C in Trout Lake during the period 1973–84. Confidence intervals were unavailable for *O. rusticus* and *O. propinquus* in 1979 and 1980.

relative size frequencies of naturally interacting populations. Nevertheless, the competition model until now has been consistent with observed changes in northern Wisconsin populations (Capelli 1982). The newer data from Trout Lake provide the first clear evidence that other ecological factors need to be considered.

Adult body size and innate aggression are the most obvious differences among species and provide a ready explanation, which is consistent with the competition model, for the increase in *O. rusticus* regardless of the population density of other species. In having both higher innate levels of aggression and a larger adult body size, *O. rusticus* has a clear competitive advantage (Capelli and Munjal 1982). For these reasons, *O. rusticus* is probably also less vulnerable to predation (see Stein and Magnuson 1976; Butler and Stein 1985) and is less restricted to substrates like cobble which provide shelter from predation (Berrill 1978; Lorman 1980; Capelli and Munjal 1982). If the decline in the population of *O. propinquus*, the middle species in the competitive hierarchy, is an independent event not caused by crayfish species interactions, then the increase in *O. virilis*, the inferior competitor, is also consistent with the competition model.

However, the dramatic declines of overall crayfish abundance (i.e. of *O. propinquus* abundance) are not consistent with the competition model, which assumes that total population levels are at an equilibrium determined by shelter availability. It is extremely unlikely that shelter availability in Trout Lake (primarily cobble) has changed appreciably. Physico-chemical conditions in Trout Lake and most other area lakes have also not changed appreciably in 50 yr (C. Bowser et al., Center for Limnology, University of Wisconsin-Madison, Madison, WI 53706, unpubl. data). Although other natural populations of crayfish have been severely depressed by disease (Unestam 1973), the effectiveness of disease has been attributed to the introduction of a new fungal vector in one case (Nylund and Westman 1983; Vey et al. 1983) and to changing environmental conditions in another (France 1983). We have seen no evidence of disease in Trout Lake.

Two other possible explanations for the decline in *O. propinquus* involve predation. The first is that the observed decline is a function of changing trapability and is not a real decline in population. Collins et al. (1983) found that the presence, type, and density of predatory fishes affected the trapability of *O. virilis* and *O. propinquus*. (*O. rusticus* was not present in the lakes studied.) We do not know if predator numbers increased in Trout Lake between 1973 and 1983, but two aspects of our results suggest that lower trap catches in 1983 were not caused by predator-induced behavioral changes. First, there were few large declines and only one significant decline in trap catches along the western shore of Trout Lake. We would expect large mobile predators like fish to have similar impact in similar habitats on both sides of the lake. Second, while *O. propinquus* catches declined, *O. virilis* and *O. rusticus* catches increased. Although predators might depress crayfish activity differently across species, trapability in all species would be depressed, not enhanced.

The second predation hypothesis is more plausible. As size-selective predators choosing the smallest crayfish prey available, smallmouth bass (*Micropterus dolomeiu*) would be more likely to reduce populations of the smallest species, *O. propinquus*, relative to *O. virilis* and *O. rusticus* (Stein 1977). *Orconectes virilis*, which is larger and probably less vulnerable to predation, would then be released from the competitive pressure of *O. propinquus*. We do not, however, have the data necessary to evaluate that hypothesis.

Although the factors involved in the changes in crayfish species populations in Trout Lake remain unknown, from the history presented, we suggest that the simple competition model is inadequate to explain the dynamics of the crayfish assemblage. Although evidence to this point (Magnuson et al. 1975; Capelli 1982; Capelli and Magnuson 1983) has suggested that crayfish species invasions are unidirectional and irreversible, the Trout Lake record shows that these patterns are variable. The invasion of Trout Lake by *O. rusticus*, which began about 1979, is proceeding slowly and if it continues, will provide us with a natural test of our predictions of benthic and fish community disturbance. Regardless of the outcome of the *O. rusticus* invasion, documented reverses in relative abundances of *O. virilis* and *O. propinquus* call for a closer examination of the role of parasitism, predation, and other forces in structuring crayfish assemblages.

Acknowledgments

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Model of Monthly Marine Growth and Natural Mortality for Babine Lake Sockeye Salmon (*Oncorhynchus nerka*)

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Furnell, D. J., and J. R. Brett. 1986. Model of monthly marine growth and natural mortality for Babine Lake sockeye salmon (*Oncorhynchus nerka*). Can. J. Fish. Aquat. Sci. 43: 999–1004.

Monthly marine growth and natural mortality rates were calculated for each major life history type of Babine Lake sockeye salmon (*Oncorhynchus nerka*) and compared with estimates by other methods. Growth rates were based on sizes at the beginning and end of each marine year and adjusted according to seasonal changes in environmental temperature. Temperature effects were derived from information on growth responses of cultured fish. Mortality estimates were based on a model which equates mortality rate to an inverse function of weight. Monthly sizes determined from the growth model were substituted into the mortality model, together with estimates of smolt and adult runs (catch plus escapement), to examine the population dynamics of an average Babine Lake smolt cohort. Using these predictions we suggest that 90% of natural mortality occurred during the first 4 mo at sea.

Nous avons calculé le taux de mortalité naturelle et le taux de croissance mensuel en mer pour chaque étape importante du cycle vital des saumons rouges (*Oncorhynchus nerka*) du lac Babine et nous les avons comparé aux estimations trouvées par d'autres méthodes. Nous avons basé les taux de croissance sur les tailles calculées au début et à la fin de chaque année passée en mer et nous les avons rajustés suivant les changements saisonniers de la température du milieu. On a tiré des conclusions sur les effets de la température à partir des informations obtenues sur la croissance de poissons d'élevage. Les estimations de la mortalité ont été basées sur un modèle qui rend le taux de mortalité égal à une fonction inverse du poids. Les tailles mensuelles déterminées à partir du modèle de croissance ont été substituées dans le modèle de mortalité, de même que les estimations des remontes d'adultes et de saumoneaux (prises plus survivants), afin d'étudier la dynamique de la population d'une cohorte moyenne de saumoneaux du lac Babine. À l'aide de ces prévisions, nous estimons que 90 % de la mortalité naturelle s'est produite pendant les quatre premiers mois de séjour en mer.

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Bioenergetics analysis of an average cohort of Babine Lake sockeye salmon (*Oncorhynchus nerka*) required monthly estimates of biomass throughout the cohort's marine life (Brett 1983, 1986). The problem was predicting the monthly natural mortality and growth rates based on sparse data. This paper reports a method for making these calculations.

Natural marine mortality estimates for sockeye salmon are complicated by an array of factors. Data have been published on the abundance of Babine Lake smolts and returning adults, which have usually spent 1, 2, or 3 yr at sea (McDonald and Hume 1984). Unfortunately, information is not available on the proportions of the smolt cohort destined to become these three major life history types. This would not be a problem if mortality were equally distributed among life history types over their marine life; however, such does not appear to be the case. Parker (1971) stated that 55–77% of a pink salmon (*O. gorbuscha*) cohort was lost to predation during its first month at sea. Mortality at this rate would soon eliminate the stock. He concluded that the smallest individuals suffered the greatest mortality. Evidence of size-dependent natural mortality can also be found in the sockeye scale analysis summarized by Ricker (1976). Within a stock, larger than average smolts of a single year-class, and year-classes of larger smolts, survive better than smaller ones; moreover, stocks with larger smolts have lower natural mortality. Because fish grow and natural mortality appears size related, a constant monthly natural mortality rate is not expected to apply throughout life.

If this is true for a single sockeye life history type, it also likely applies to comparisons between the 3_2, 4_2, and 5_2 types (3_2 refers to a fish that spends its first year growing in freshwater, migrates to sea in its second year, and returns to spawn in its third year, living all or part of two growing seasons in the ocean). More than 98% of Babine sockeye migrate to sea after 1 yr in freshwater, and only these were considered. There is strong evidence of higher growth rates among earlier maturing life history types (Lander and Tanonaka 1964). Therefore, it is probable that the different life history types experience different rates of natural mortality.

The problem of assigning different mortality rates to each life history type can be resolved if a single unifying assumption is made: that natural mortality is a function of size (i.e. weight). The function we used was a simple inverse propor-

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TABLE 1. Estimates of weight, W (grams), and annual instantaneous marine growth rates, g (where $g = \text{Log}_e(W2/W1)$) for Babine Lake sockeye salmon. The first annual rate applies to the first 14 mo of marine life.

Life history type	First year (14 mo)			Second year (12 mo)			Third year (12 mo)		
	$W1$	$W2$	g	$W1$	$W2$	g	$W1$	$W2$	g
3_2	5.35[a]	774[b]	4.9745		N/A			N/A	
4_2	5.35	550[c]	4.6328	550	2285[d]	1.4246		N/A	
5_2	5.35	550	4.6328	550	1369[c]	0.9119	1369	2919[d]	0.7572

[a]McDonald and Hume (1984, table 5).
[b]Forester (1968, table 100).
[c]Lander and Tanonaka (1964, table 13).
[d]Clemens (1947, table XI).

tionality as adopted by Mathews and Buckley (1976) for coho salmon (*O. kisutch*). Their analysis was, however, limited by the assumption that all life history types have identical, constant growth rates. Ricker (1976), referring to the approach as "objective and intuitively reasonable," applied an inverse weight–mortality relationship to the complex growth pattern of a single Karluk sockeye life history type using an estimated total mortality (Fredin 1964). The approach (described under Mortality Model) produces its own total natural mortality estimate for each life history type. It extends Ricker's tabular method to any number of life history types, each with complex, dissimilar growth curves, and enables back-calculation of the proportions of a smolt cohort which become each life history type. The method requires estimates of size-at-age for all life history types, the initial number of smolts, and the resulting runs of each adult type.

Growth Model

A primary requirement for employing an inverse relationship between mortality and size is a growth model which gives reasonable predictions of weight with time at sea. The model we used recognized the different growth rates of the three life history types of a Babine sockeye salmon cohort (Table 1). In the first year, two groups of fish were separated: those destined to become 3_2 adults and the balance of fish which return as 4_2 and 5_2 adults. Individual instantaneous annual growth rates were calculated for each life history type. Growth rates for the 3_2 type were based on an initial mean smolt weight and the average size of adults returning after 14 mo. Rates for 4_2 and 5_2 fish used the same initial smolt weight and the mean size of immature fish taken in the Gulf of Alaska after 14 mo at sea (Lander and Tanonaka 1964). The mean size of immature fish at 14 mo was assumed to apply to potential 4_2 and 5_2 fish, as it was impossible to distinguish between these two types at this age. In the second marine year, two groups were again identified: maturing 4_2 fish and slower growing 5_2 salmon. Growth rates for 4_2 sockeye were based on the size of immature Gulf of Alaska fish after 14 mo at sea and the mean size of returning Babine Lake 4_2 adults 12 mo later. The growth rates for 5_2 fish were derived from the size of immature Gulf of Alaska fish after 14 and 26 mo at sea (Lander and Tanonaka 1964). The instantaneous growth rate for maturing 5_2 sockeye in their final marine year was derived from the weight of immature Gulf of Alaska sockeye after 26 ocean mo and the size of returning 5_2 Babine Lake adults. This procedure gave five different annual instantaneous growth rates each corresponding to a specified stage of the different life history types (Table 1).

Seasonal variation in growth rates was estimated by incorporating bioenergetics data concerning the temperature effects on sockeye growth with data on annual temperature changes. During their marine life, sockeye salmon encounter average daily temperatures ranging from 4.5 to 12.5°C on a known seasonal cycle (Brett 1983, table 1 and fig. 6). Temperature has a complex effect on fish growth, influencing the amount eaten and the metabolic rate. The growth rates of wild sockeye salmon exceed those of cultured specimens on maximum rations (Brett 1983), and it is a fair assumption that wild fish at sea are feeding near their optimum level. The effect of temperature on the growth of cultured sockeye on maximum rations has been determined by Brett (1974, table V). For all weights examined, the relationship between instantaneous growth rate and temperatures between 4.5 and 12.5°C was linear ($r^2 > 0.98$ in all cases). This indicated a direct proportionality between growth rate and average daily temperatures normally experienced at sea.

Unfortunately, the relationships between growth rate and temperature, determined by Brett (1974), could not be applied directly to wild fish which grow more rapidly and reach larger sizes than those examined experimentally. This required scaling the linear temperature–growth relations of cultured fish to intersect the mean growth rate and temperature experienced by wild fish (Fig. 1). Scaling by retaining either the slopes or the intercepts of cultured fish relationships (Fig. 1) did not, however, produce satisfactory results. If the slopes were retained, excessively high growth rates occurred at low temperatures where little growth was expected (Fig. 1). Conversely, if the intercepts were retained, the relationships became improbably steep. The model selected here (Fig. 1, lines A–E) was intermediate between these extremes. Lines A–E (Fig. 1) fulfilled the growth requirements of Table 1 and were intermediate in slope and intercept to the scaling options discussed above. The resulting growth model assumed no growth at 0°C and a linear relationship between instantaneous growth rate and temperature over the range of temperatures experienced by wild sockeye.

For brevity, calculation procedures are demonstrated in Table 2 for the 3_2 life history type only. The annual growth rates of Table 1 were all partitioned similarly using the temperature data of Brett (1983). The growth curve resulting from this model, applied to all life history types, illustrates a seasonal slowing of growth during winter (Fig. 2). This slowing would be more pronounced if growth rate–temperature intercepts

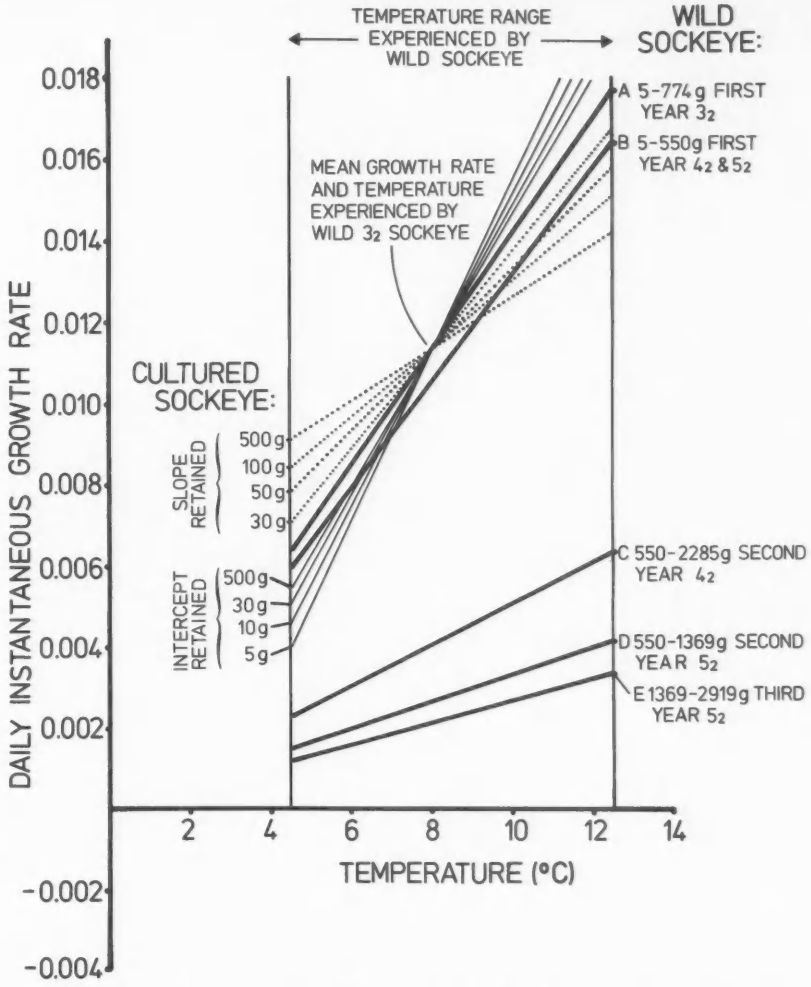

FIG. 1. Comparison of growth rate – temperature relationships for cultured sockeye salmon with those used for wild fish (lines A–E). To illustrate the intermediate nature of the model, relations for cultured fish have been made to pass through the mean growth rate and temperature experienced by wild 3_2 fish while retaining either the slopes (dotted lines) or intercepts (fine lines) of the original relations presented by Brett (1974).

from cultured fish were retained. Retaining slopes would de-emphasize seasonal changes. The only obvious anomaly occurred when immature fish began their second marine year. The earliest growth rates (months 15 and 16) were considerably lower than for the previous month, although no marked temperature decrease was experienced. This was an artifact of having to begin use of the second year's growth rate in August when the 14-mo-old, 550-g immature 3_2 fish were sampled in the Gulf of Alaska. The effect of this anomaly on mortality estimates was minimal, however, as the rate of natural mortality had declined to low levels by this stage of life.

Mortality Model

Estimates of the smolt run [$N(t = 0)$] can be separated into components each representing the number of smolts producing one life history type (three for Babine Lake sockeye (McDonald and Hume 1984)); thus:

$$(1)\quad N(t=0) = Na(t=0) + Nb(t=0) + Nc(t=0)$$

where: N = number of fish; $a = 3_2$ life history type, $b = 4_2$ life history type, $c = 5_2$ life history type, and t = time (months). Each component can be estimated from the abundance of the adult runs, such that

$$(2)\quad Na(t=0) = Na(t=n1)e^{\sum_{t=1}^{n1} Ma(t)}$$

$$(3)\quad Nb(t=0) = Nb(t=n2)e^{\sum_{t=1}^{n2} Mb(t)}$$

$$(4)\quad Nc(t=0) = Nc(t=n3)e^{\sum_{t=1}^{n3} Mc(t)}$$

TABLE 2. Estimates of the monthly instantaneous growth rate at sea of 3_2 life history type Babine Lake sockeye salmon adjusted for monthly average temperatures (°C) given by Brett (1983, table 1 and fig. 6).

t	Month end	Mean surface temperature (T)	Monthly growth rate $(T/\Sigma T)4.9745$
0	May	—	—
1	June	11.0	0.4762
2	July	12.0	0.5195
3	August	12.5	0.5412
4	September	11.0	0.4762
5	October	9.0	0.3896
6	November	6.5	0.2814
7	December	4.8	0.2078
8	January	4.5	0.1948
9	February	4.5	0.1948
10	March	4.5	0.1948
11	April	5.3	0.2295
12	May	7.3	0.3160
13	June	9.5	0.4113
14	July	12.5	0.5412
		Sum = 114.9 = ΣT	Sum = 4.9745

where $n1$ = the last month of marine life of the 3_2 type, $n2$ = the last month of marine life of the 4_2 type, $n3$ = the last month of marine life of the 5_2 type, and $M(t)$ = monthly instantaneous mortality rate. The relative magnitude of the different monthly rates is an inverse function of weight; hence:

(5) $Ma(t) = f/Wa(t)$

(6) $Mb(t) = f/Wb(t)$

(7) $Mc(t) = f/Wc(t)$

where W = weight (grams) and f = constant. Substituting equations 5, 6, and 7 into 2, 3, and 4, respectively, and the results into equation 1 produces the following expression:

$$N(t=0) = Na(t=n1)e^{\sum_{t=1}^{n1} f/Wa(t)} + Nb(t=n2)e^{\sum_{t=1}^{n2} f/Wb(t)} + Nc(t=n3)e^{\sum_{t=1}^{n3} f/Wb(t)}.$$

This equation was solved iteratively for f by computer and natural mortality rates of Babine Lake sockeye estimated from equations 5, 6, and 7. The iterative procedure involved inserting trial values of f until the final equation was balanced with appropriate significant figures. Values for $N(t=0)$, $Na(t=n1)$, $Nb(t=n2)$, and $Nc(t=n3)$ were obtained from McDonald and Hume (1984, table 13). Values for $Wa(t)$, $Wb(t)$, and $Wc(t)$ were derived from the previously described growth model. Applying this model to each of the 1961–67 preenhancement mean smolt and adult runs reported by McDonald and Hume (1984) shows that roughly 90% of mortality occurs during the first 4 mo at sea (Fig. 3). The standard errors of the mean monthly mortality rates for years 1961–67, due to annual variation, were in all cases approximately 7% of the estimate.

Discussion

Perhaps the most complete, concise summary of sockeye salmon mortality rates is given by Ricker (1976, table 9). Many of these estimates do not distinguish the different life history types or examine the temporal distribution of mortality. Those unbiased estimates meeting at least one of the above criteria are compared with our estimates (Table 3). Natural mortality rates calculated here are generally higher during early marine life and lower during later months than estimates by other methods. The mortality comparison and tabular inverse weight methods of Ricker (1976) are the most directly comparable because of their temporal distribution within a specified life history type. Both these estimates are based on Karluk 5_3 sockeye with much larger smolts (20–30 versus 5.35 g) and smaller adults (1620–1680 versus 2285 g) than Babine Lake sockeye. If

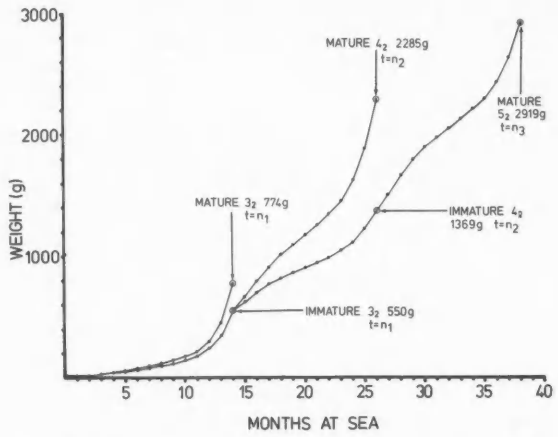

FIG. 2. Change in weight of average Babine Lake sockeye salmon over their marine life. Growth rates used to develop these relationships were derived by monthly partitioning of annual growth according to mean monthly water temperatures (t = time in mo; $n1$, $n2$, and $n3$ = last marine month for 3_2, 4_2, and 5_2 fish, respectively).

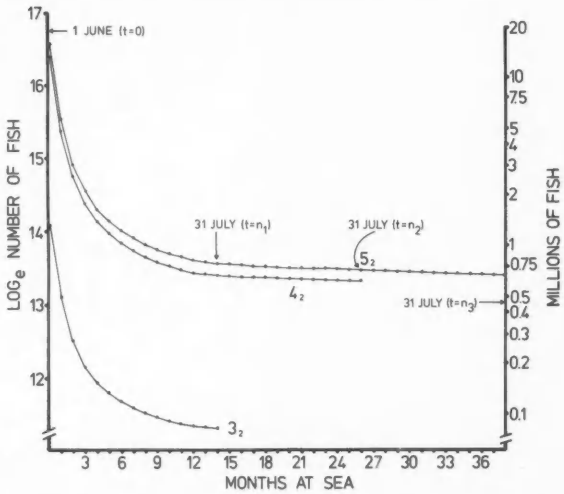

FIG. 3. Change in numbers of an average Babine Lake sockeye smolt cohort over its marine life. Monthly instantaneous mortality rates are equal to the difference between adjacent points on the natural logarithmic scale.

TABLE 3. Comparison of our sockeye mortality rates with those presented by Ricker (1976, table 9). Monthly rates have been averaged on an annual basis where necessary to make comparison possible.

Method	Life history type	Mortality period	Mean instantaneous monthly mortality rate	Range of mortality rates
Comparison of life histories	5_2	Final 12 mo	0.014	N/A
Ocean immatures	5_2 and 6_3	Final 12 mo	0.018	N/A
Inverse weight	5_3	First 12 mo	0.095	0.382–0.024
		Final 12 mo	0.014	0.022–0.008
Mortality comparison	5_3	First 12 mo	0.052	0.195–0.039
		Final 12 mo	0.019	0.039–0.000
This study	3_2	14 mo sea life	0.198	0.998–0.011
	4_2	First 14 mo	0.214	1.022–0.016
	4_2	Final 12 mo	0.008	0.013–0.004
	5_2	First 14 mo	0.214	1.022–0.016
	5_2	Second 12 mo	0.010	0.014–0.006
	5_2	Final 12 mo	0.004	0.006–0.003

the assumption of an inverse relationship between mortality and size is correct, the difference in mortality estimates is as expected.

This model is subject to two obvious sources of data error: (1) incorrect estimates of smolt or adult runs and (2) inaccurate weights at the beginning and end of each year. Consequently it is important to examine the sensitivity of the model to these variables. Full stochastic sensitivity analysis of the model is not currently warranted without more information on the probability density distributions of the parameters or confirmation that the inverse size mortality relationship is valid. Hence a simple perturbation sensitivity analysis has been applied. Errors in estimating adult run size in a single life history type affect estimates of mortality for that type as well as the other life history types (Table 4). Overestimating a single adult run decreases its mortality and that of the other life history types. The reverse is true for underestimates of single adult runs. If all adult runs are overestimated, all mortality rates are negatively biased; underestimates have the opposite effect. Overestimating smolt runs positively biases mortality rates; underestimates cause a negative bias.

The mortality rates presented were calculated assuming equal size smolts for all life history types (Table 1) as expected for fish spending the same time (approximately 1 yr) in freshwater. It is, however, believed that the largest smolts are more likely to mature earlier (Foerster 1968). The effect of negative errors in 3_2 smolt weight estimates is a decrease in mortality rates for the 3_2 type and an increase for the others (Table 4). Adult sizes can be influenced by the type of sampling gear. Overestimating adult weight reduces mortality rates for the life history type involved and increases them for the others. Underestimates have the reverse effect.

The evidence reviewed by Parker (1971), Mathews and Buckley (1976), and Ricker (1976) strongly suggests that a negative size mortality relationship exists. The existence of such a relationship with unknown form should be an impetus for future research. To assume an inverse relationship is perhaps simplistic and ignores the hidden assumptions of similar natural mortality agents operating both inshore and offshore,

TABLE 4. Sensitivity of the model to errors in estimates of smolt and adult run sizes of all life history types and errors in estimates of weight for 3_2 smolts and adults. Total mortalities are the sum of all monthly estimates over the entire marine life.

Parameter change	3_2 total mortality	4_2 total mortality	5_2 total mortality
Actual	2.773	3.083	3.157
Increase smolt run 20%	2.936	3.263	3.343
Decrease smolt run 20%	2.574	2.861	2.931
Increase 3_2 run 20%	2.765	3.074	3.149
Decrease 3_2 run 20%	2.781	3.091	3.166
Increase 4_2 run 20%	2.698	2.999	3.072
Decrease 4_2 run 20%	2.855	3.174	3.251
Increase 5_2 run 20%	2.685	2.985	3.058
Decrease 5_2 run 20%	2.870	3.191	3.268
Increase all adults 20%	2.610	2.902	2.972
Decrease all adults 20%	3.128	3.477	3.562
Increase 3_2 smolt weight 20%	2.441	3.095	3.170
Increase 3_2 adult weight 20%	2.640	3.088	3.163
Decrease 3_2 adult weight 20%	2.900	3.077	3.151

the effect of size-selective offshore fishing, or possible seasonal change in natural mortality patterns. We have adopted this relationship primarily for illustrative purposes and believe the mortality–size relationship requires detailed investigation. Further complexity in this relationship should be incorporated into equations 5, 6, and 7. Natural and inshore fishing mortality might also be separated at this level. Our model assumes that temperature is the dominant factor influencing sockeye growth. This is true for captive sockeye on maximum rations; however, the situation is likely far more complex for wild fish. Simple empirical data could help determine this. These refinements would result in better definition of the temporal patterns of growth and mortality in wild sockeye.

To summarize, the model presented produces reasonable estimates of marine natural mortality for sockeye salmon, based only on smolt and adult abundance and growth. It avoids the problem of arbitrarily assigning mortality over time or

between life history types. The method depends on an inverse relationship existing between weight and natural mortality for which there is strong inferential evidence. Establishing the precise form of this relationship is clearly an important focus for improving understanding of the natural mortality experienced by sockeye salmon.

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Factors Influencing the Microdistribution of Two Sympatric Species of Plecoptera: An Experimental Study

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The responses of nymphs of two species of perlid stoneflies to manipulation of some important features of their microhabitats were studied in laboratory stream tanks fitted with an infrared beam interruption system. The activity of both *Paragnetina media* (Walker) and *Phasganophora capitata* (Pictet) was significantly affected by current speed and substrate size, and by an interaction between the two. Regression analysis showed that activity for *P. capitata* was highest at 15 and 75 cm/s and minimal between 25 and 50 cm/s, while that for *P. media* was highest at 15 cm/s and lowest at 25 cm/s and above. For *P. media* there was a significant negative relationship between substrate size and activity at a current speed of 15 cm/s. The relationship between the activity of *P. capitata* and substrate size was not linear. Addition of trout skin mucus to the stream tanks caused an increase in the levels of activity of *P. media* but a decrease in the activity of *P. capitata*. Addition of nymphs of the mayfly *Ephemerella subvaria* to the tank containing *P. media* produced no effect. These findings suggest mechanisms that establish different microdistributions for sympatric species.

On a étudié dans des réservoirs à écoulement continu de laboratoire auxquels on avait adapté un système d'interruption par faisceau infrarouge les réactions qu'ont eues des nymphes de deux espèces de plécoptères de la famille des perlidés suite à la manipulation de certains éléments importants de leur microhabitat. La vitesse du courant et la taille du substrat, de même qu'une interaction entre les deux, ont beaucoup influé sur l'activité de *Paragnetina media* (Walker) et de *Phasganophora capitata* (Pictet). L'analyse de la régression a montré que dans le cas de *P. capitata*, l'activité était la plus grande à des vitesses de 15 et 75 cm/s et minimale entre 25 et 50 cm/s, tandis que pour *P. media* elle était la plus grande à 15 cm/s et la plus faible à 25 cm/s et à des vitesses supérieures. Pour ce qui est de *P. media*, il y avait une relation négative importante entre la taille du substrat et l'activité lorsque la vitesse du courant était de 15 cm/s. La relation entre l'activité de *P. capitata* et la taille du substrat n'était pas linéaire. L'addition de mucus provenant de la peau de truites aux réservoirs à écoulement continu a fait augmenter le degré d'activité de *P. media* mais a fait diminuer celui de *P. capitata*. L'addition de nymphes de l'éphémère *Ephemerella subvaria* au réservoir contenant *P. media* n'a eu aucun effet. Ces résultats portent à croire qu'il y a des mécanismes qui déterminent des microdistributions différentes pour les espèces sympatriques.

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The microdistribution of stream benthos is controlled by many factors. They include substrate type, water currents, food quality and abundance, light, temperature, water chemistry, oviposition habits, and predation. Some of these parameters may act singly but many act in combination (Hynes 1970).

It has been argued that substrate is perhaps the primary factor in creating microhabitats, especially as it is correlated with many of the other factors, for example current speed (Williams 1981). Williams (1980) suggested that the degree of association with a particular substrate mixture varies from species to species; it may be a primary association in some species but secondary or even tertiary in others. Water currents, too, appear to control microdistribution, as studies on net-spinning caddisflies have shown distinct preference ranges in different species (Edington 1968; Boon 1978). However, water currents are difficult to quantify accurately, especially in the field, as substrate particles create complex microcurrents and stagnant boundary layers (Trivellato and DeCamps 1968) and the technology to measure these is lacking.

Minshall (1984, p. 388) pointed out that "the study of aquatic insect – substratum relationships has progressed to a relatively high level of refinement compared with other areas of aquatic insect ecology." He added, however, that to be able to produce useful generalizations, more detailed information is needed on such topics as the effects of substrate particle size and the role of plant matter within the substrate. Studies on named species will make this information of greater value. Recently, Williams and Moore (1985b) have addressed the role of epilithon in substrate selection by stream invertebrates. The influence of other biotic factors such as predator or prey species may be important also (Peckarsky 1984; Williams and Moore 1985a).

The present study is an attempt to examine, in the laboratory where environmental conditions can be carefully controlled, the responses of nymphs of two sympatric species of perlid stoneflies to manipulation of some important abiotic and biotic features of their microhabitats. These features were substrate particle size, current speed, and the presence of predatory fish or mayfly prey. Based on the findings of these experiments, it

TABLE 1. Summary of ANOVA applied to transformed data (log $y + 1$) on activity levels of *P. media* (M) and *P. capitata* (C) at five current speeds on two sizes of substrate.

Source of variation	df	Sum of squares		Mean square		*F*-value		Prob. > *F* M and C
		M	C	M	C	M	C	
Substrate size	1	0.56	1.61	0.56	1.61	13.83	15.89	<0.001
Current speed	4	2.52	3.53	0.63	0.88	15.44	8.70	<0.001
Substrate × current	4	6.12	6.38	1.53	1.60	37.46	15.74	<0.001
Error	110	4.49	11.15	0.04	0.10			
Total	119	13.70	22.67					

may be possible to infer the factors separating the microhabitats of "co-occurring" species.

Materials and Methods

The stonefly species studied were *Paragnetina media* (Walker) and *Phasganophora capitata* (Pictet). Both are common in eastern North America where they occur in a wide variety of streams (Hitchcock 1974). Specimens for this study were collected from Duffin Creek, Durham County, Ontario (43°58′N, 79°05′W). This is a third-order, hardwater stream, the features of which have been described by Williams (1980). Collections were made frequently from October to April of 1983–85 but only nymphs greater than 15 mm long were retained. These were held in large, well-aerated tanks in a controlled environment room at 12°C for 2 wk prior to testing. The photoperiod of the room was reversed (i.e. dusk in the environment room fell at 11 a.m. EST), for the convenience of the experimenter, but the nymphs very quickly resynchronized their activity patterns and levels. Food was supplied in the form of a live mixture of other benthic species from Duffin Creek, especially mayflies, hydropsychid caddisflies, and chironomids.

Responses of the nymphs to manipulation of their microhabitat were studied as changes in activity level (measured as numbers moving per hour) in recirculating, moulded, fibreglass stream tanks of 43-L capacity (2068-cm^2 bottom area). This assumes that organisms move around less in microhabitats that they "prefer" and are more active in less suitable surroundings, the result of simple kinetic or taxic responses. Activity levels were monitored automatically using an infrared beam interruption technique. This technique has been used successfully for the amphipod *Gammarus pseudolimnaeus* Bousfield (Wallace et al. 1975; Williams and Moore 1982) for which an ultraviolet light beam was used. However, aquatic insects are able to detect ultraviolet light, and the system was modified for infrared which they cannot see. Output from the photocell was recorded on a chart recorder in the form of one vertical ink line for each time a nymph passed through the beam.

For each experiment, 40 large nymphs of one species were placed in the stream tank in a depth of 13 cm of water. The nymphs were allowed to acclimate to the tanks for 18 h before recording began; no food was supplied in the experimental tanks but the nymphs had been fed previously. As the nymphs were primarily nocturnally active (see Fig. 4), only nighttime activity data were used in the analysis. Each experiment was replicated, using a fresh batch of nymphs, until at least 12 h of activity had been obtained per experimental condition. The tanks were cleaned carefully between trials. The following microhabitat manipulations were done for each of the two stonefly species.

(1) *Variation of current speed*. For this, the nymphs were placed on a scrubbed, uniform substrate (screened gravel, 11.0 mm in diameter) and activity was recorded at currents of 0, 15, 25, 50, and 75 cm/s. This experiment was repeated using the same range of current speeds but over a larger-sized substrate (60.0 mm in diameter).

(2) *Variation of substrate size*. Here, the tanks were set to run at a constant current speed of 15 cm/s and successive batches of nymphs were tested on four different substrate sizes: sand, <1.0 mm in diameter; small gravel, 11.0 mm; large gravel, 60.0 mm; and cobbles, >150 mm. (In Duffin Creek, nymphs of both species are found in areas where the substrate ranges from 11.0 to >150 mm in diameter and where current ranges from approximately 15 to >75 cm/s. Densities of nymphs appear to vary greatly over these ranges but this has not been quantified.)

(3) *Presence of a predator*. Skin mucus was scraped from several rainbow trout (*Salmo gairdneri*) and added (as a suspension in 500 mL of water) to the tanks 3 h after artificial sunset at which time the nymphs' activity was high. Control levels of stonefly activity were monitored prior to each addition, and trials using plain water ensured that the nymphs were not just responding to novel stimuli.

(4) *Presence of prey*. Here, 300 large nymphs of the Duffin Creek mayfly *Ephemerella subvaria* McDunnough were added in a small plastic cage. This prevented them from interfering with the light beam and from physical contact with the stoneflies. This addition was done for *P. media* only.

Trials with both predator and prey were done under standard microhabitat conditions of 13-cm water depth over 11.0-mm-diameter gravel at a constant current speed of 15 cm/s. All experiments were done under conditions of 12 h light : 12 h dark at 12°C.

Results

For both *P. media* and *P. capitata*, current speed over the range 0–75 cm/s significantly affected the activity of the nymphs (ANOVA, $p < 0.001$) (Table 1). Similarly, for both species, change from a substrate size of 11.0 to 60.0 mm significantly affected activity ($p < 0.001$). In addition, there was a significant interactive affect of current speed and substrate size on nymph activity ($p < 0.001$).

To determine the nature of these relationships, linear and curvilinear regressions were applied to current speed and activity, and substrate size and activity, for each species. Although there is a significant ($p < 0.05$), negative linear correlation between current speed and nymphal activity for *P. media* on 11.0-mm-diameter substrate, application of polynomial regression to the activity data for 15, 25, 50, and 75 cm/s (excluding the still-water trials which may sometimes produce unusual

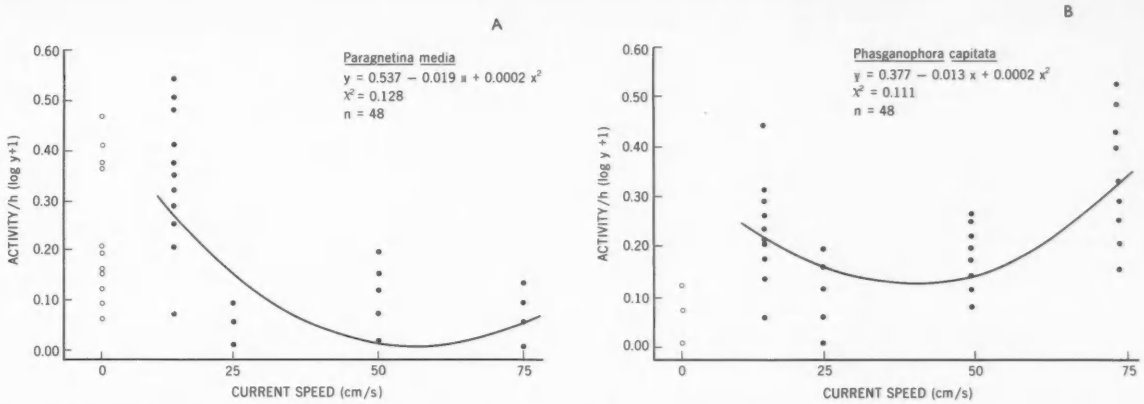

FIG. 1. Relationship between the activity of (A) *Paragnetina media* and (B) *Phasganophora capitata* and current speed on 11.0-mm substrate. There are 12 values of y for each value of x, but in some cases on the graph it appears less, as some points are superimposed on others; still-water data are indicated by the open circles and were not included when fitting the lines.

behaviour in lotic animals; Elliott 1967) provides a better fit (Fig. 1A) ($\chi^2 = 0.143$ for linear regression; $\chi^2 = 0.128$ for the polynomial regression[1]; derived from Datagraphics Statistical Package, Scientific Microprograms, Raleigh, NC). The polynomial regression is described by the equation $y = 0.537 - 0.019x + 0.0002x^2$. The line indicates that nymphal activity was highest at 15 cm/s and was lower at speeds of 25 cm/s and above.

The relationship between current speed (excluding the still-water data) and nymphal activity for *P. capitata* on 11.0-mm-diameter gravel is curvilinear, described by the quadratic equation $y = 0.377 - 0.013x + 0.0002x^2$ (χ^2 goodness of fit = 0.111; $p > 0.9$). It indicates that nymphal activity was high at 15 and 75 cm/s and minimal somewhere between 25 and 50 cm/s.

On substrate of mean diameter = 60.0 mm there were no significant relationships between current speed and nymphal activity (*P. media*, $r^2 = 0.000$; *P. capitata*, $r^2 = 0.075$).

For *P. media*, at a current speed of 15 cm/s, nymphal activity decreased with increasing substrate size (Fig. 2); nymphs were most active on sand and least active on cobbles. The relationship is described by the quadratic regression equation $y = 0.904 - 0.371x + 0.039x^2$ (χ^2 goodness of fit = 0.11; $p > 0.9$).

Regression of the activity of nymphs of *P. capitata* on different substrate sizes produced no biologically meaningful relationships (linear regression proved to be nonsignificant, $r^2 - 0.245$, and polynomial regression produced relationships that involved negative values of activity in that the curve dipped below the x-axis). Plots of the raw data, however, clearly show that nymphs were more active on sand and small gravel substrates than on large gravel and cobbles (Fig. 3).

Addition of fish (rainbow trout) skin mucus to the stream tanks caused a significant increase in the level of activity of

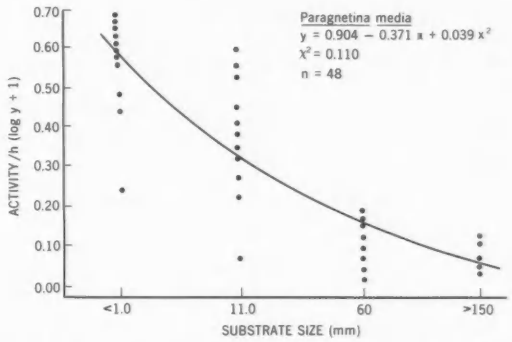

FIG. 2. Relationship between the activity of *Paragnetina media* and substrate size. Substrate classes are shown equally spaced. There are 12 values of y for each value of x, but in some cases on the graph it appears less, as some points are superimposed on others.

P. media ($p < 0.01$) and a decrease in the level of activity of *P. capitata* (Table 2) (see Discussion). Addition of 300 caged nymphs of the mayfly *E. subvaria* to the tank containing *P. media* produced no effect.

Discussion

Clearly, both current speed and substrate size had a significant effect on the activity of the two species of stoneflies and some of this effect was interactive. Relationships between nymphal activity and current speed were evident only on 11.0-mm-diameter gravel and not on large gravel (60.0 mm in diameter). Regression of substrate size against nymphal activity was significant and negative for *P. media* and removed some 75.6% of the variance in activity. Although some relationship between substrate size and activity existed for *P. capitata*, it could not be meaningfully described by regression analyses.

Some of the unexplained variance in activity may have been due to the physiological state of the nymphs, as some experiments spanned several weeks. Within such a time frame, microhabitat selection of nymphs might reasonably be expected

[1]The χ^2 value represents a measure of how individual data points in a class set (observed distribution) deviate from the mean (i.e. the point through which the line is drawn, which equals the predicted distribution). It is thus a better evaluation of the fit of the line to the data points than considering the mean deviation as is done when using sum of squares (Bevington 1969); H_0 = there is a good fit of the data to the curve; probability of rejecting H_0, in this case, is >0.9.

TABLE 2. Changes in levels of activity of stonefly nymphs in response to biotic additions to the activity tanks.

Stonefly species	Addition factor	Activity level (no./h) Before addition ($\bar{x}$ ± 1 SE) (n)[a]	After addition ($\bar{x}$ ± 1 SE) (n)[a]	Prob.
Paragnetina media	Trout mucus	0.18 ± 0.06 (74)	0.61 ± 0.13 (36)	<0.01[b]
Phasganophora capitata	Trout mucus	0.39 ± 0.09 (92)	0.22 ± 0.11 (36)	>0.5[b], <0.05[c]
Paragnetina media	*Ephemerella subvaria*	0.41 ± 0.16 (34)	0.34 ± 0.13 (32)	>0.5[b]

[a]These values refer to hourly totals of activity prior to and after addition; each addition was repeated between 4 and 6 times.
[b]Probability of a significant difference between the two sets of activity levels, from a Mann-Whitney *U*-test.
[c]Probability of a significant difference from a Wilcoxon paired-sample test.

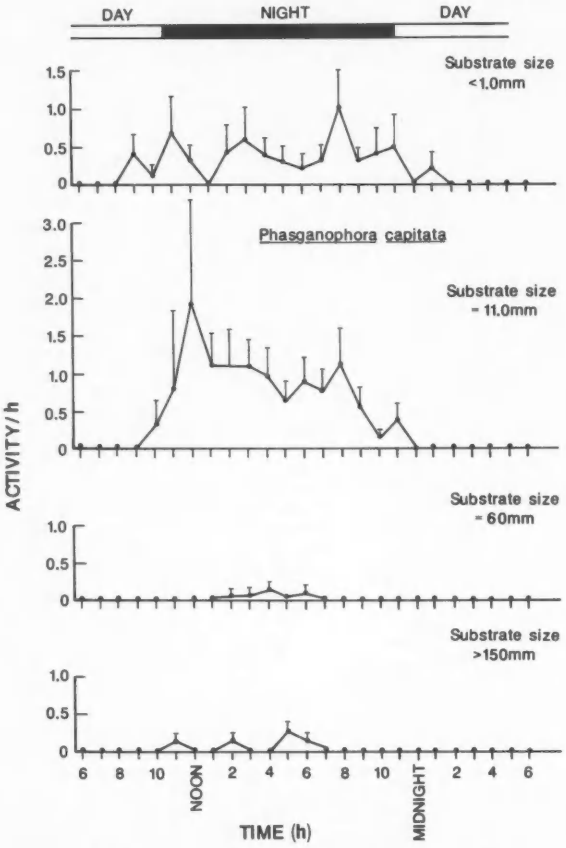

FIG. 3. Plots of hourly activity over 24 h on different sizes of substrate at 15 cm/s for *Phasganophora capitata*. Points on the graph represent the mean values and single error bars (+1 SE) are shown. For <1.0-mm substrate, n = 10; for 11.0-mm substrate, n = 8; for 60-mm substrate, n = 20; for >150-mm substrate, n = 8).

to change as the requirements of the individual change (see Pringle 1982; Ciborowski and Clifford 1983). For example, nymphs that are normally associated with fast currents and large substrate particles necessarily have to move towards the banks and into areas of slower current and smaller particles prior to emergence of the adult. Again, change from one instar to the next may be accompanied by a change in diet which may necessitate a change in microlocation.

Addition of fish skin mucus to the activity tanks produced a significant increase in the activity of *P. media*. Detection of this stimulus by *P. media* implies that these nymphs were capable of receiving some information from their microenvironment via detection of waterborne chemicals. This has been shown for *G. pseudolimnaeus* (Williams and Moore 1985a) in which case the active component was a relatively nonlabile, high molecular weight fraction of fish mucus. In *Gammarus*, the animals' response was a decrease in activity as they stopped drifting and settled on the substrate. In *P. media*, the response was one of increased crawling between the substrate particles. One interpretation of this phenomenon might be that they were seeking improved hiding places. The addition of trout mucus to *P. capitata* had no significant effect on nymphal activity, as shown by the Mann-Whitney *U*-test (Table 2). However, analysis of these data by the Wilcoxon paired-sample test, which allows for seasonal changes in the data, showed that postaddition activity was significantly ($p < 0.05$) lower. Experiments on the addition of mucus to *P. capitata* were the only ones of those given in Table 2 that lasted for several weeks. There may, therefore, have been some temporal effect on activity, as alluded to earlier. The direction of change in activity seen in *P. capitata* was opposite to that seen in *P. media* and seems to indicate a different response to trout predators.

Both *P. media* and *P. capitata* eat *E. subvaria* in nature (D. D. Williams, pers. observ.). Despite this, *P. media* failed to respond, in terms of a change in activity level, to the presence of a high density of these mayflies in their tank. It seems likely, therefore, that long-distance chemical cues (or perhaps even auditory ones) are not involved as stimuli in interactions between them and this particular prey species. Although the experiment was not done with *P. capitata*, direct observations of interactions between *P. capitata* and *E. subvaria* strongly suggest two components to the predatory behaviour of *P. capitata*: (1) physical contact with the prey by the predator's antennae and (2) a chemical that is detectable only over distances of a few millimetres (D. D. Williams, unpubl. data).

In conclusion, the data show some similarities and some differences in response to manipulation of microhabitat features between these two sympatric species of stoneflies. For example, *P. media* was less active at current speeds of 25 cm/s and higher on small gravel, while *P. capitata* was least active at current speeds between 25 and 50 cm/s. However, both species were less active on larger substrates compared with sand and small gravel at a current speed of 15 cm/s. Both species, then,

would seem to favour microhabitats featuring large substrate particles, but *P. media* selects a wider range of currents than *P. capitata*. This may indicate a mechanism for establishing different microdistributions and possibly also niche separation, within small areas of streambed. As the differential response to current speed by the two species was not apparent on the 60.0-mm substrate, the results emphasize the extreme complexity of the effects of microhabitat features on single species. Even in the laboratory, under carefully controlled conditions, much of the variance in the activity data was unaccounted for. How much more difficult is the task of deciphering these relationships on entire benthic communities in the field? Clearly, there is scope for much more work to be done on this topic.

Acknowledgments

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Cortisol, Aldosterone, and Leucocytes in the Stress Response of Bottlenose Dolphins, *Tursiops truncatus*

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The activity associated with capturing, restraining, and removing bottlenose dolphins, *Tursiops truncatus*, from water stimulates a stress response as reflected by circulating cortisol, aldosterone, and eosinophils. Serum cortisol increased from resting levels of about 30 to 110 nmol/L within 1 h, aldosterone rose from less than 280 pmol/L to up to 1880 pmol/L within 3 h, and circulating eosinophils were depressed to less than 40% of their initial numbers within 7 h after the animals were removed from water (calm-capture). This basic response was not enhanced when the capture procedure was prolonged for 3 h (chase-capture) or when the dolphins were given adrenocorticotropic hormone and was similar to that observed in free-ranging dolphins after they had been held in a net for up to 5 h. Eosinophil numbers appear to be a consistent and practical indicator of stress in dolphins.

Le fait de capturer, de retenir et de retirer de l'eau les dauphins à gros nez, *Tursiops truncatus*, est cause d'un stress qui se traduit par une variation des teneurs en cortisol, aldostérone et eosinophiles circulants. On a noté, par rapport aux teneurs au repos, une augmentation de la concentration de cortisol qui est passée d'environ 30 à 110 nmol/L en moins d'une heure et de celle de l'aldostérone qui est passée de moins de 280 à 1 880 pmol/L en moins de 3 h. Quant aux éosinophiles circulants, leur taux s'est abaissé à moins de 40 % de la valeur initiale en moins de 7 h après la sortie de l'eau des animaux (capture sans poursuite). Cette réponse de base n'a pas été intensifiée par l'extension à 3 h de la procédure de capture (poursuite–capture) ou par l'administration d'hormone adrénocorticotrope; la réponse demeurait semblable à celle, observée après maintien dans un filet, de dauphins libres, pendant une période pouvant atteindre 5 h. Il semble que le dénombrement des éosinophiles soit un indicateur uniforme et pratique du stress chez les dauphins.

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Stress in terrestrial mammals can be monitored by measuring elevations in serum concentrations of adrenal corticosteroids (Dantzer and Mormede 1983), particularly cortisol. As a partial effect of increased hormone levels, the total number of circulating neutrophils becomes elevated, while lymphocytes and eosinophils decrease (Gwazdauskas et al. 1980).

Bottlenose dolphins, *Tursiops truncatus*, appear to exhibit the same changes, but to a lesser extent than do terrestrial mammals. Medway and Geraci (1966), in a study on hematology, found that dolphins stressed for 36 h exhibited eosinopenia and mild neutrophilia, which they attributed to an adrenocortical response. Later, Medway et al. (1970) found that administration of dexamethasone, a synthetic glucocorticoid, induced eosinopenia and lymphopenia but not neutrophilia. The reason for the inconsistent responses is not clear because the degree of stress was not controlled, nor was it quantified using adrenal steroids as a measure.

As a first step toward understanding these relationships, we undertook this three-part study to determine the nature of the stress response in *Tursiops*. We examined the effects of handling and of adrenocorticotropic hormone (ACTH; corticotrophin) on adrenocortical hormones, circulating leucocytes, and on selected serum constituents influenced by exertional stress in dolphins (Colgrove 1978; Geraci and Medway 1973).

Our plan was to capture and then restrain bottlenose dolphins for 6 h out of water, with the degree of prior manipulation as a variable. To do this, we used two capture techniques: one involved corralling dolphins with a net for 3 h (chase-capture), and the other was performed as quickly and with as little herding as possible (calm-capture). Their response to each capture regime was then compared with that in dolphins given ACTH. We also examined the levels of circulating cortisol and aldosterone in free-ranging *Tursiops*.

Materials and Methods

Collection of Blood Samples

In June 1983, we determined the stress response associated with capturing and handling three dolphins (numbered 1, 2, and 3, Table 1). The animals had been held in captivity for 3 yr during which they were handled frequently for other behavioral and physiological studies. The dolphins were maintained in 20 × 20 × 1.5 m deep sea-pens at the Institute for Delphinid Research (IDR), Grassy Key, FL. Air and water temperatures ranged from 29 to 33°C and from 30 to 33°C, respectively, and the salinity was 35‰. Each dolphin was fed an average daily diet of 1 kg of thawed smelt, *Osmerus mordax*, and 5 kg of thawed herring, *Clupea harengus*, which provided 2.5×10^5 J·kg body wt^{-1}·d^{-1}. The animals were fasted 12 h prior to each test day.

TABLE 1. Characteristics of 10 captive bottlenose dolphins (*Tursiops truncatus*) used in the capture and ACTH stimulation studies.

Dolphin No.	Sex	Age (yr)	Body weight (kg)
1	F	4	140
2	F	4	123
3	F	4	165
4	M	8	190
5	M	5	200
6	M	10	200
7	F	10	200
8	F	9	200
9	M	15	190
10	M	9	170

To capture a dolphin, we corralled it into a small area of the sea-pen using a net. The animal could then be restrained manually around the thorax and placed in a stretcher. Each dolphin served as its own control. On one test day, it was captured within 10 min using as little herding as possible (calm-capture). It was then placed in a stretcher, moved 30 m to a shaded carrier frame, and kept wet for up to 6 h. On another day, the same dolphin was separated from the others and for 3 h was repeatedly chased, corralled, and released. It was then captured and handled in the same prescribed way (chase-capture). To minimize the possible bias associated with the first capture experience, we used two different testing sequences. Dolphins 1 and 3 were first chase-captured and then calm-captured 3 d later, whereas dolphin 2 was first calm-captured and then chase-captured 6 d later.

On each test day, blood samples were obtained just before the dolphin was removed from water (within 10 min of capture), after it was secured in the carrier frame (about 15 min after removal from water), and 30 min and 1, 3, and 6 h thereafter. A final sample was obtained when the dolphin was returned to the sea-pen, just before it was released from the stretcher.

Blood for hematology was collected from the flukes or dorsal fin (Geraci and Sweeney 1978) by syringe, or Vacutainer assembly (Becton Dickinson and Co., Rutherford, NJ), placed in tubes containing EDTA as the anticoagulant, and stored at 25°C before analysis. Eosinophils were counted within 24 h and total leucocytes were determined within 48 h. These storage times have no appreciable effect on white blood cell numbers (Geraci and Medway 1974; Thomson 1984). Blood for serum was collected in tubes without anticoagulant, and within 4 h of collection was centrifuged or 20 min at 750 × *g*. Serum was separated and frozen at −20°C for up to 4 mo before being analyzed.

Four days after the conclusion of the capture study, two of the three dolphins (Nos. 1 and 3) were used to assess the effects of ACTH on adrenocortical hormones and leucocyte numbers. The third animal was excluded because it had refused food for 3 d. As a substitute, we retested dolphin 1 in October 1983. Each dolphin was captured as quickly as possible with the least amount of disturbance and was held out of water under precisely the same conditions as in the previous study. The first blood sample was collected as soon as the animals were secure in the carrier frame. Within 20 min they were given, intramuscularly, 50 IU of Cortrosyn (Organon Canada Ltd., Toronto, Ont.), a synthetic subunit containing 24 of the amino acids of natural ACTH. Blood samples were then collected variably at 15 and 30 min and at 1, 1.5, 3, 3.5, 4, and 5 h after injection. The dolphins were then returned to water. Dolphin 1 was recaptured and sampled the following day in both the June and October studies.

Early results of the June experiment indicated that Cortrosyn elicited only a mild adrenocortical response. To determine if this was due to the type of preparation used, we compared the response of dolphins to Cortrosyn with that to ACTHar, a natural porcine corticotrophin (Harris Laboratories, Toronto, Ont.).

In this study, conducted in October 1983, we used seven other dolphins (Nos. 4−10, Table 1) which had been in captivity for 3−9 yr, during which they had been used in a modest show schedule and were rarely handled. Three of the animals (Nos. 4, 5, and 6) were maintained in sea-pens at IDR. The others were held in a 1.5-m-deep pentagonal sea-pen with a surface area of 300 m^2 at Flipper's Sea School, Key West, FL.

We used a similar protocol to that in the previous ACTH stimulation trials. Four dolphins were given ACTHar and three were given Cortrosyn, all at a dose of 50 IU. For logistic reasons, the time required to capture the dolphins was generally longer (up to 2 h) than in the handling study; and the animals were also held in the stretchers for a longer time (up to 2 h) before initial blood samples were collected and ACTH was administered.

During the course of this experiment, we were able to obtain blood samples from free-ranging bottlenose dolphins at the time of their capture. This provided us with the opportunity to compare the results of our controlled manipulation study with that of animals at sea following herding and capture. Single blood samples were collected from 38 animals immediately after they were held in a net for up to 5 h and then hoisted onto a raft.

Analytical Procedures

Serum constituents were analyzed in duplicate. Cortisol was determined by radioimmunoassay (New England Nuclear, Boston, MA), modified to include 30 nmol/L standards. Maximum binding of ^{125}I tracer to antiserum was 62 ± 9% (n = 27; C.V. = 15%), and nonspecific binding of tracer to blank antiserum and samples was 3.4 ± 0.5% (n = 25; C.V. = 15%). Inter- and intra-assay variations were 15.8% (n = 36) and 6.8% (n = 10), respectively. Cortisol values of diluted samples were an average of 97% of those for undiluted samples (n = 8; C.V. = 15.5%). Sensitivity of the assay was 10 nmol/L.

Serum aldosterone concentrations were determined by radioimmunoassay (InterMedico, Willowdale, Ont.). Inter- and intra-assay variations were 15.1% (n = 16) and 15.6% (n = 10), respectively. Values for diluted samples were 102% (n = 6; C.V. = 8%) of those for undiluted samples, indicating linearity. Mean recovery of aldosterone standard from samples was 95% (n = 22; C.V. = 16.9%). Maximum binding of ^{125}I tracer to antiserum was 47 ± 5% (n = 20; C.V. = 10%), and nonspecific binding of tracer was 1.8 ± 0.9% (n = 19). Sensitivity of the assay was 50 pmol/L.

Serum glucose levels were analyzed using Sigma test kit No. 15-UV (Sigma Chemical Co., St. Louis, MO). Inter- and intra-assay variations were 11.4% (n = 11) and 8.0% (n = 10), respectively.

Serum creatine kinase (CK) and alanine aminotransferase (ALT) concentrations were assayed using kinetic test kits (Boerhinger Mannheim GmbH, Mannheim, West Germany). Interassay variation was 10.6% for CK (n = 14) and 10.7% for ALT (n = 6). Intraassay variation was 4.2% for CK

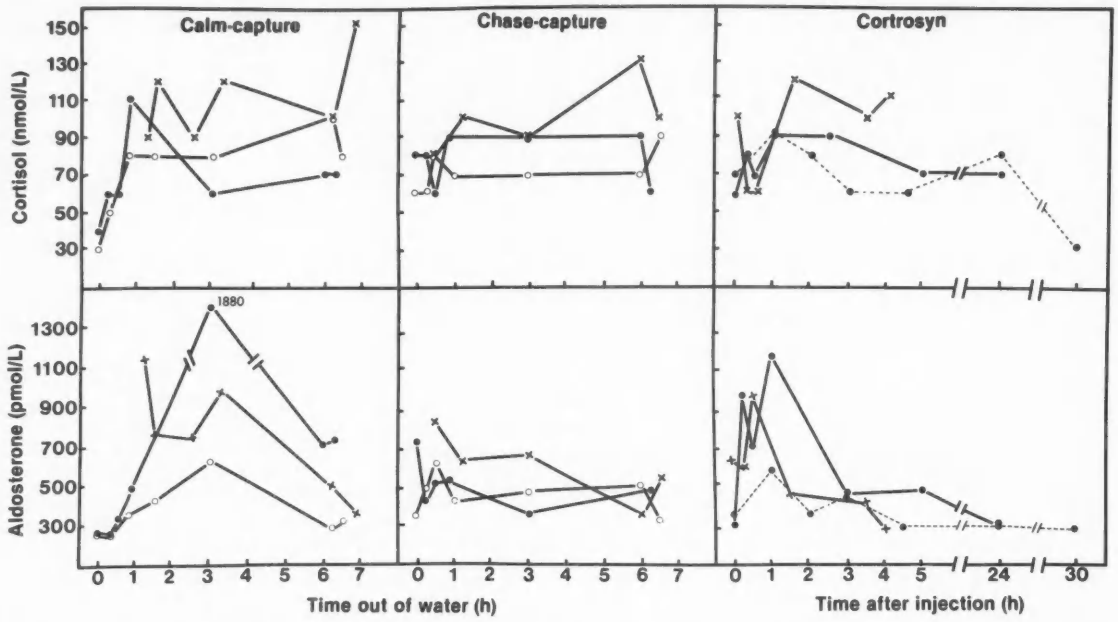

FIG. 1. Serum cortisol and aldosterone concentrations in three dolphins following calm-capture, chase-capture, and after they were given 50 IU synthetic ACTH (Cortrosyn). ACTH administration did not increase cortisol beyond that already stimulated by handling. ●—●, dolphin 1 in June; ●---●, dolphin 1 in October; ○, dolphin 2; ×, dolphin 3.

(n = 10) and 8.1% for ALT (n = 7).

Serum sodium (Na) and potassium (K) ion levels were analyzed using flame photometry (Bach-Simpson Ltd., London, Ont.). Intra- and inter-assay variabilities were less than 8% (n = 5).

Total leucocytes (WBC) and eosinophils were counted directly in duplicate or triplicate using hemocytometers and Unopettes (Becton Dickinson and Co., Rutherford, NJ). Intra-assay variation was 15% (n = 10) for leucocyte counts and 20.3% (n = 11) for eosinophil counts. Dried blood smears were stained with Wright's stain and 500 leucocytes were examined to determine the relative number of each cell type. Total lymphocyte values were calculated from these percentages by multiplying by the total leucocyte count.

Results

Because we used results of the calm-capture and chase-capture study as controls for the initial Cortrosyn stimulation trials, data for these studies are shown together in Fig. 1 and 2.

All three animals remained docile throughout both study protocols, except dolphin 3 which tended to squirm each time it was placed in a stretcher. Therefore, the first blood sample was collected only after this animal had been out of water for 75 min following calm-capture and 30 min after chase-capture.

Following calm-capture, initial serum cortisol concentrations were 30 and 40 nmol/L in dolphins 1 and 2 (Fig. 1). During the first hour out of water, cortisol values rose abruptly to 80−120 nmol/L and generally remained within this range for the remaining 6 h. Aldosterone in these animals showed the same general trend, beginning at less than 280 pmol/L, rising to a range of 650−1880 pmol/L by 3 h, with a return to 290−750 pmol/L by 6 h.

Immediately after chase-capture, cortisol levels were 100% higher (dolphins 1 and 2) than at the same period after calm-capture; in both groups, cortisol eventually stabilized within a similar range (60−100 nmol/L). Following chase-capture, serum aldosterone remained constant within the range of 300−700 pmol/L. There was only a mild increase in cortisol values after Cortrosyn was administered, and values generally ranged between 60 and 110 nmol/L thereafter.

The aldosterone response to Cortrosyn was consistent in all three trials (Fig. 1). Values were initially 300−630 pmol/L, peaked at 580−1170 pmol/L within 1 h, and then declined to less than 500 pmol/L after 3 h. Dolphin 1 had a more pronounced response in June than in October.

Initial eosinophil numbers for dolphins 1, 2, and 3 were 0.7×10^9, 3.1×10^9, and 1.2×10^9/L, respectively, during calm-capture and 0.8×10^9, 1.6×10^9, and 0.8×10^9/L, respectively, following chase-capture (Fig. 2). In the Cortrosyn trials, initial values for dolphins 1 and 3 in June and 1 in October were 0.6×10^9, 1.1×10^9, and 1.2×10^9/L, respectively. In all cases, numbers began to decrease within 1 h and a decline was still apparent up to 7 h later. By the following day, eosinophil values in dolphin 1 during both Cortrosyn trials had recovered to their original levels.

Initial lymphocyte numbers for dolphins 1, 2, and 3 during calm-capture were 1.3×10^9, 1.8×10^9, and 0.6×10^9/L, respectively, and during chase-capture were 1.8×10^9, 0.9×10^9, and 0.5×10^9/L, respectively (Fig. 2). The lymphocyte response following Cortrosyn administration was not consistent. Beginning at initial values of 1.7×10^9, 1.4×10^9, and 0.7×10^9/L in dolphin 1 in June, 1 in October, and 3, respectively, numbers uniformly decreased within 2 h. Thereafter, including the following day, lymphocyte counts fluctuated within or below the pretest range.

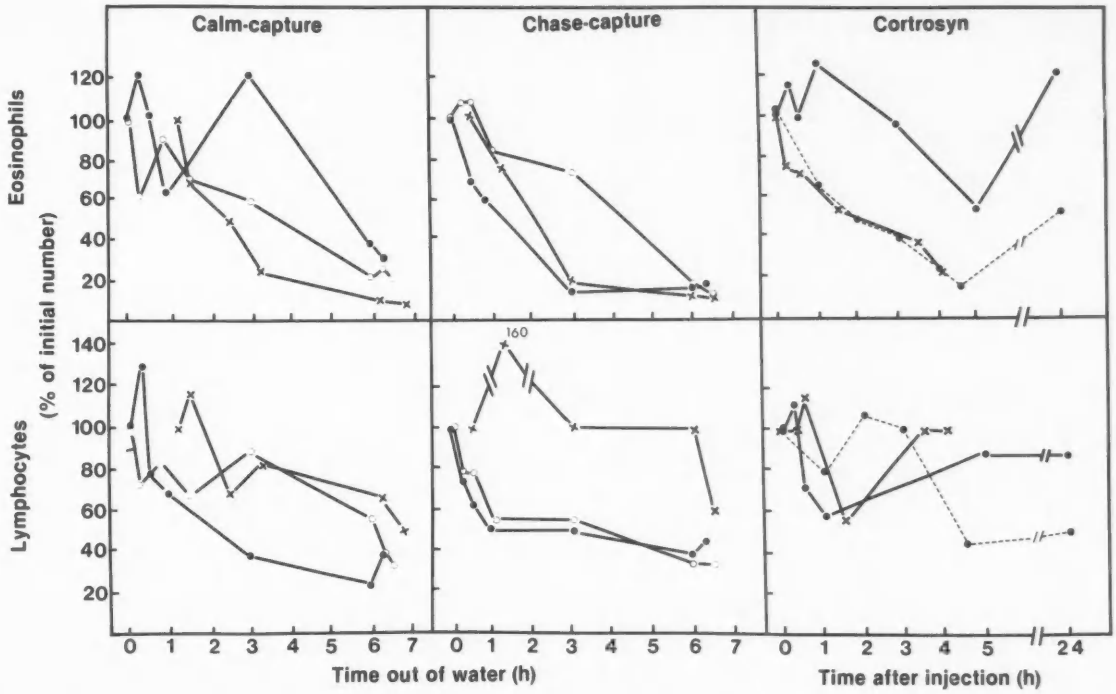

FIG. 2. Changes in circulating eosinophils and lymphocytes in three dolphins following calm-capture, chase-capture, and after they were given 50 IU synthetic ACTH (Cortrosyn). Eosinopenia was uniform in all three dolphins during all protocols whereas lymphopenia occurred only during calm- and chase-capture. Symbols as in Fig. 1.

TABLE 2. Means, standard deviations, and ranges of some serum chemical constituents which were not affected by calm-capture, chase-capture, or ACTH administration in 10 captive bottlenose dolphins (*Tursiops truncatus*) (n = 90).

Serum constituent	$X \pm$ SD	Range
CK (U/L)	45±20	12–119
ALT (U/L)	14±6	7–38
Na (mmol/L)	155±4	149–160
K (mmol/L)	3.9±0.4	3.2–5.6
Glucose (mmol/L)	8.5±2.1	5.6–16.6

The patterns of change in cortisol, aldosterone, and eosinophils were similar in the dolphins given Cortrosyn and those injected with ACTHar (Fig. 3). Circulating lymphocyte numbers were not affected.

As in the earlier trials, Cortrosyn elicited a peak aldosterone response within 1 h, which decreased to pretest levels within 4 h. The ACTHar preparation induced a similar change in two of the four dolphins tested. Values for serum CK, ALT, Na, K, and glucose were not affected by any of the study protocols (Table 2).

Two dolphins died after they were given synthetic or natural ACTH. Dolphin 3 was found dead 48 h after the injection of Cortrosyn. Necropsy revealed advanced autolysis, and therefore the tissues could not be histologically examined. The lungs, lymph nodes, and adrenal glands were congested and edematous. The pterygoid sinuses were inflamed and contained trematodes (*Nasitrema* sp.). Dolphin 5 refused food 3 d after it was given ACTHar. It appeared lethargic by the fifth day, when it died minutes after it was removed from water for examination. Gross necropsy revealed mild pancreatic duct fibroplasia, prescapular lymphadenitis, and hemorrhagic enteritis. Histologically, hepatic and adrenal congestion and focal bronchiolitis were also observed.

Serum cortisol (100 ± 30 nmol/L, n = 38) and aldosterone (370 ± 270 pmol/L, n = 27) concentrations in the free-ranging dolphins were similar to those of captive dolphins given ACTH and to those following calm- and chase-capture.

Discussion

The activity associated with capturing, restraining, and removing dolphins from water elicited a stress response which made it difficult to determine "resting" concentrations of cortisol and aldosterone. We found the lowest values for both steroids in two dolphins within 10 min after calm-capture, the same time frame used to establish resting levels in other captive wildlife (Van Heerden and Bertschinger 1982). If we assume these to represent basal values, then resting cortisol concentrations of 30–40 nmol/L are comparable with those of other mammals, including dogs (Vial et al. 1979) and rabbits, *Sylvilagus floridanus* (Hamilton and Weeks 1985). The lowest aldosterone values of 280 pmol/L are also similar to resting levels in other mammals (Armbruster et al. 1975; Kajihara et al. 1983). Interestingly, pinnipeds, the only other group of marine mammals for which information is available, have

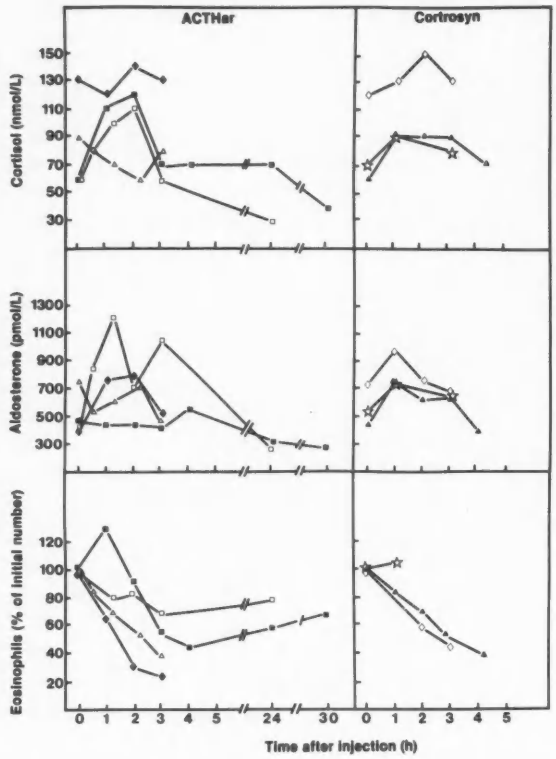

FIG. 3. Serum cortisol and aldosterone concentrations and changes in circulating eosinophil numbers in seven dolphins given 50 IU natural (ACTHar) or synthetic (Cortrosyn) ACTH. The preparations resulted in no consistent changes in either steroid but both were equally effective in reducing eosinophil numbers. □, dolphin 4; ■, dolphin 5; ☆, dolphin 6; △, dolphin 7; ▲, dolphin 8; ◆, dolphin 9; ◇, dolphin 10.

considerably higher resting concentrations of aldosterone (Sangalang and Freeman 1976; Malvin et al. 1978; Englehardt and Ferguson 1980), for reasons which are not understood.

A stress response was evoked even under the calmest conditions of capture we could employ. By 1.5 h after calm-capture, cortisol concentrations had peaked and stabilized between 80 and 120 nmol/L. In the animals which were chased, the peak response had probably already occurred by the time they were captured and sampled, as all value during their 6-h period out of water were between 60 and 100 nmol/L. These changes represent up to fourfold elevations over estimated basal values, and are comparable with those associated with herding in reindeer, *Rangifer tarandus* (Rehbinder and Edqvist 1981) and handling in Alaskan moose, *Alces alces gigas* (Franzmann et al. 1975).

Neither corticotrophin preparation elevated cortisol beyond levels induced by manipulation and removal from water. Typically, in mammals given ACTH, cortisol increases three- to four-fold above pretest levels (Paape et al. 1977; Hamilton and Weeks 1985). We considered several possible explanations for our observations. Perhaps cortisol is not an adequate indicator of adrenal stimulation in dolphins, and, as in rodents (Kaneko et al. 1981), another steroid such as corticosterone would be more useful. The preparations may have been inappropriate or the dosages insufficient. As a fourth alternative, the lack of response may have reflected the dolphins' inability to serete, because the adrenal cortex may already have been maximally stimulated.

We believe cortisol is, in fact, a good indicator of adrenal activity in dolphins. Seal and Doe (1965) found that dolphin transcortin has equivalent affinity to cortisol and corticosterone. Following that theme, we analyzed serum from 32 free-ranging *Tursiops* and found that cortisol predominates over corticosterone in a ratio of 5:1.

We are also satisfied that the preparations were suitable and the dosage sufficient. In light of our initial experiments, we had been concerned that Cortrosyn might not have been an appropriate stimulus, even though it shares the same basic amino acid sequence with other mammals, including whales (Kawauchi et al. 1978). For that reason, we included ACTHar in our protocol and administered both preparations at dosages that were equivalent to or greater than those used to elicit a clear response in other species, including deer (Van Mourik and Stelmasiak 1984), horses (Hoffsis et al. 1970), cows (Pappe et al. 1977), and the beluga whale, *Delphinapterus leucas* (U. S. Seal, F. Wright, E. D. Plotka, and A. McDevitt. Baseline serum hormones and their response to hypothalamic releasing hormones in two beluga whales. Presented at the 11th Annual Conference of the International Marine Animal Trainers Association 1983). Yet there was still no consistent response.

This lack of response was probably influenced by pre-existing cortisol levels already elevated by handling. The only dolphins which showed a pronounced response were Nos. 4 and 5, which had lower pretest levels before being given ACTHar. They showed only a twofold increase, peaking at levels which were similar to those observed in both calm- and chase-captured animals. All dolphins with high pretest levels showed little or no response. Others have also demonstrated an inverse correlation between basal serum cortisol and its response to corticotrophin in mammals (Vial et al. 1979; Hermus et al. 1984). The mechanism is unknown, but it has been suggested that in subjects in which basal levels are high, endogenous ACTH may have already reduced the cortisol reserves of the adrenal gland so that its response to exogenous stimulation is blunted (Kukreja and Williams 1981). Our findings suggest that, in these dolphins, the adrenal cortex may have been stimulated during the course of handling to the extent that it could not be further stimulated with exogenous ACTH.

It is curious that dolphins which have been in captivity for many years, and are handled routinely, elicit a significant stress response when manipulated. The response appears to be the same in free-ranging dolphins. Based on the cortisol values we obtained, the adrenal cortex of those animals also seems to have been stimulated by the time they were captured and sampled.

We have no way of gauging the implications of such a sensitive response or its adaptive value. How might a stimulated animal react to superimposed stressors? Perhaps the death of two dolphins given ACTH provides a clue. Our examination of the mammalian literature of the last 30 yr has revealed no similar occurrence. Perhaps dolphins exhibit a unique sensitivity to stimulation by this hormone, therefore limiting our ability to conduct further studies. Alternatively, stimulation of the adrenal in the face of preexisting stress may, under some conditions, be detrimental, and perhaps this type of sensitivity

accounts for often fatal consequences associated with capturing and confining certain species, such as *Phocoena phocoena* (Dudok van Heel 1962), *Phocoenoides dalli* (Ridgway 1966), and *Delphinis delphis* (Walker 1975). However unlikely they are to be conducted, studies on stress using these species might reveal the basis underlying their sensitivity to capture and confinement.

Aldosterone responded to all the protocols in essentially the same way as did cortisol, with some important differences. Unlike cortisol, aldosterone generally showed a significant peak within 1 h after injection of Cortrosyn or ACTHar, indicating that it had not yet reached its maximum level as a result of handling. After calm-capture, aldosterone rose from low basal values to a peak response within 3 h, thereafter declining. The chase-captured dolphins showed quite a different response, with levels of aldosterone remaining low throughout, indicating that they may have already peaked and subsided by the time the animals were captured. These values may be viewed as a chronological extension of those of the calm-captured animals. The combined findings of these studies point to the influence of corticotrophin on aldosterone secretion in dolphins. As in other mammals, there is dual control, the other being the renin–angiotensin system (Malvin et al. 1978). It would be interesting to investigate the relative influence of both systems in the dolphin, which depends so vitally on electrolyte and water balance in the marine environment.

Among the other blood constituents examined, we focused on two types of leucocytes: lymphocytes and eosinophils. Lymphocytes, through the redistributional action of cortisol, can be significantly reduced to the extent of compromising an animal's immune response (Parillo and Fauci 1979). Our calm-capture studies show that when a dolphin is captured and removed from water, lymphocyte numbers can decrease by 50% between 3 and 7 h but not in dolphins given ACTH. Although influenced by stress, lymphocytes in dolphins appear to have limited value as a reliable index of the stress response itself. In contrast, eosinophils show particular sensitivity to stress. In all protocols, cell numbers decreased immediately at a rate which continued throughout the time the animals were restrained. Eosinophil counts showed a gradual recovery in two animals (Fig. 3) and a near complete recovery in two others (Fig. 1) by the following day. These findings provide quantitative support of earlier suggestions that circulating eosinophils are an index of stress in dolphins (Medway and Geraci 1966; Medway et al. 1970), probably associated with the redistributional action of glucocorticoids (Parillo and Fauci 1979). Eosinophil counting would seem to be a useful and practical tool to assess stress in captive animals for which baseline values can be established through routine monitoring. Because eosinophils defend the body against parasites (Tavassoli 1981), and stress reduces their numbers, it would be interesting to determine the limits of this balance. At what point can ongoing stress reduce these cells below their capacity to protect?

In conclusion, we examined some aspects of the adrenal stress response in dolphins. Routine handling procedures appeared to stimulate a cortisol response which was not enhanced by additional stimuli, such as exogenous ACTH or prolonged, aggressive capture. The cortisol elevation is temporally and consistently associated with an immediate and steady decline in eosinophils. We suggest that eosinophil counting is a useful and practical tool to assess the extent and duration of stress in dolphins.

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Physiological Responses to Acid Stress in Crayfish (*Orconectes*): Haemolymph Ions, Acid–Base Status, and Exchanges with the Environment

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Wood, C. M., and M. S. Rogano. 1986. Physiological responses to acid stress in crayfish (*Orconectes*): haemolymph ions, acid–base status, and exchanges with the environment. Can. J. Fish. Aquat. Sci. 43: 1017–1026.

Exposure of *Orconectes propinquus* for 5 d to pH = 4.0 (H_2SO_4) in decarbonated soft water ($[Ca^{2+}]$ = 0.20 mequiv·L^{-1}) caused a severe metabolic acidosis and a moderate depression of $[Na^+]$ and $[Cl^-]$ in the haemolymph. Lactate did not accumulate. Acidosis was caused by a large uptake of acidic equivalents from the environmental water, of which more than 95% was stored outside the extracellular compartment after 5 d. Carapace buffering was probably involved, because haemolymph $[Ca^{2+}]$ rose substantially and Ca^{2+} was lost to the environment. Similar net effluxes of K^+ indicated that acidic equivalents also penetrated the intracellular compartment. SO_4^{2-} was also lost during acid exposure. Haemolymph $[Na^+]$ fell more than $[Cl^-]$ because of greater net losses to the water. Unidirectional flux analyses with radiotracers demonstrated that negative net Na^+ and Cl^- balance resulted from partial inhibition of influx components; effluxes were little affected. All flux effects were reversed during 5 d of recovery at pH = 7.5. Haemolymph ionic responses in *Orconectes rusticus* differed in showing a smaller, equimolar reduction of $[Na^+]$ and $[Cl^-]$ and a much larger elevation of $[Ca^{2+}]$. At a mechanistic level, the responses of crayfish to acid stress appear very different from those of teleost fish.

L'exposition pendant 5 jours d'*Orconectes propinquus* à une eau douce décarbonatée ($[Ca^{2+}]$ = 0,20 méquiv·L^{-1}) de pH (H_2SO_4) de 4,0 s'est traduite par une acidose métabolique sévère accompagnée d'une baisse modérée de la teneur en ions Na^+ et Cl^- de l'hémolymphe. Il n'y a pas eu accumulation de lactate. L'acidose résultait d'une importante entrée d'équivalents acides à partir de l'eau du milieu et l'on retrouvait plus de 95 % de ceux-ci emmagasinés à l'extérieur du compartiment extracellulaire après 5 jours. La carapace exerçait probablement un effet tampon, car la teneur en Ca^{2+} de l'hémolymphe s'est élevée de façon appréciable et il y a eu perte de Ca^{2+} dans le milieu. Des pertes nettes semblables de K^+ indiquaient que des équivalents acides avaient aussi pénétré dans le compartiment intracellulaire. Du SO_4^{2-} a aussi été perdu au cours de l'exposition au milieu acide. La teneur en Na^+ de l'hémolymphe s'est abaissée plus que celle en Cl^- suite à des pertes nettes dans l'eau plus importantes. Des analyses par radiotraceurs du transport unidirectionnel vers l'eau ont permis de démontrer que l'équilibre net négatif du Na^+ et du Cl^- résultait d'une inhibition partielle des composantes d'entrée, celles de sortie étant peu modifiées. Tous ces effets sur le transport ont été inversés au cours d'une période de récupération de 5 jours à un pH de 7,5. Les réponses ioniques de l'hémolymphe étaient différentes chez *Orconectes rusticus* en ce que l'on notait une réduction équimolaire des teneurs en Na^+ et en Cl^- moins importante et une augmentation beaucoup plus importante de la teneur en Ca^{2+}. Les mécanismes de réponse de l'écrevisse au stress acide semblent différer de beaucoup de ceux notés chez les poissons téléostéens.

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The physiology of acid stress in teleost fish has been intensively studied, and it is now clear that the gill is the primary site of toxic action (cf. Wood and McDonald 1982; McDonald 1983a; Howells 1984 for recent reviews). Effects on branchial O_2 uptake, acid–base balance, and ionoregulatory mechanisms have been described, with the latter predominating as the cause of toxicity at environmentally realistic levels of acid stress (i.e. pH's $\geq$ 4.0). Crustacean communities are severely affected by environmental acidification (e.g. Leivestad et al. 1976; Singer 1982; France 1983) but physiological responses have received scant attention. Crayfish, however, are a notable exception; effects on Na^+ uptake (Shaw 1960b), postmoult calcification (Malley 1980), haemolymph oxygenation (Järvenpää et al. 1983), and haemolymph ionic and acid–base status (Morgan and McMahon 1982; McMahon and Morgan 1983) have been described. Only one study was done in soft (i.e. low $[Ca^{2+}]$) water (Malley 1980). In the wild, acidification is exclusively a softwater problem, and the fish literature indicates that $[Ca^{2+}]$, the major component of water hardness, has a critical modifying effect on both acid toxicity and the nature of the toxic syndrome (McDonald et al. 1980; McDonald 1983a, 1983b).

Our goal was to assess the physiology of acid stress in crayfish under softwater conditions resembling those in affected regions of eastern Canada where stream pH's may fall as low as 4.0 during snowmelt or rainstorms (e.g. Jeffries et al. 1979; Harvey and Lee 1982). To facilitate comparisons with recent studies on fish under the same conditions (McDonald et al. 1980; Wood and McDonald 1982; McDonald et al. 1983; McDonald 1983b; Höbe et al. 1984), similar techniques for evaluating branchial function and internal regulation have been employed. These include repetitive sampling of haemolymph

for ionic, acid–base, and lactate status, the latter as an index of O_2 transport disturbance, and measurement of the unidirectional exchanges of Na^+ and Cl^- and the net fluxes of acidic equivalents and other ions between the animal and its environment. We chose the crayfish *Orconectes propinquus* because the species is endemic to the acid-threatened region of eastern Canada (Berrill 1978); we used *Orconectes rusticus*, which has been widely introduced throughout the same area, for supplementary measurements because of its larger size.

Materials and Methods

Experimental Animals and Water

Experiments were performed on 130 adult intermoult *O. propinquus* (4.2 ± 0.9 g; $\bar{X}$ ± 1 SE) collected by dipnet from Spencer's Creek, Dundas, Ontario, and 12 intermoult *O. rusticus* (17.1 ± 1.8 g) purchased from Boreal Laboratories, Mississauga, Ontario. Identification was performed by the keys of Crocker and Barr (1968). The animals used did not moult for at least 3 wk prior to and 2 wk following the experiments. For both species, the origins were hard water, as animals of soft water origin were unavailable in sufficient numbers. The composition of the hard water was as follows: $[Ca^{2+}] \cong 1.8$; $[Na^+] \cong 0.6$; $[Cl^-] \cong 0.8$; $[Mg^{2+}] \cong 0.3$; $[K^+] \cong 0.05$; $[SO_4^{2-}] \cong 0.5$; titration alkalinity ≅ 2.0 mequiv·L^{-1}; pH ≅ 8.0. In the laboratory, the animals were first adjusted to experimental temperature (10 ± 1°C) in hard water for 1 wk and then transferred to vigorously aerated artificial soft water for a further 2–3 wk. The loading rate was 0.5 g·L^{-1}, and the water was changed weekly. The animals were fed commercial trout pellets and furnished with pieces of black ABX pipe as refugia to minimize cannibalism. The soft water was prepared as a 1:10 dilution of hard water with distilled water, decarbonated by acidification to pH = 2.8 with H_2SO_4, aerated for 24 h, and then titrated with KOH and NaOH back to the appropriate pH (control = 7.5; acid = 4.0). The composition was as follows: $[Ca^{2+}] \cong 0.20$; $[Na^+] \cong 0.20$; $[Cl^-] \cong 0.15$; $[Mg^{2+}] \cong 0.03$; $[K^+] \cong 0.20$; $[SO_4^{2-}] \cong 0.25$; titration alkalinity ≅ 0.20 mequiv·L^{-1}. One week prior to experimentation, the required crayfish (6–12) were transferred to a smaller tank containing 60 L of freshly made soft water and ABX pipe refugia and thereafter starved to minimize any influence of feeding history on the experimental results. Two days prior to experimentation, each crayfish received a numbered tag. In those animals intended for arterial haemolymph sampling, a small hole was drilled through the carapace directly above the pericardial sinus and sealed with several layers of dental dam and cyanoacrylate glue so as to create a sampling port.

The small size of *O. propinquus* limited the amount of haemolymph which could be withdrawn. Total haemolymph volume is ~0.28 mL·g body weight^{-1} (Kerley and Pritchard 1967). For this reason, only the largest specimens (5–10 g) were used in the haemolymph sampling experiments, and an identically sampled set of control animals were run at neutral pH in all series. The larger *O. rusticus* (14–20 g) were employed in an experiment (series iii) requiring the daily withdrawal of a much larger sample (200 μL) for ionic measurements. All samples were drawn quickly (10–20 s) and with minimal disturbance into ice-cold gas-tight Hamilton syringes while the animal was underwater in its experimental tank. The sampling needle was fitted with a cuff permitting only 2-mm penetration to prevent damage to internal organs.

Experimental Series

(i) Haemolymph acid–base status was assessed on a control day followed immediately by 5 experimental days (repetitive sampling) in *O. propinquus* either kept at pH = 7.5 (n = 9) or exposed to pH = 4.0 (n = 11). A final measurement was taken on day 12. On day 0, haemolymph samples (50 μL) were drawn from the animals in their acclimation/starvation tanks at pH = 7.5; the crayfish were then transferred to identical tanks at either pH = 7.5 or 4.0 for subsequent sampling on days 1–5 and 12. The water was changed on days 5 and 10. Water pH was checked twice daily and adjusted as necessary by addition of 1 *M* KOH or 0.5 *M* H_2SO_4; fluctuations were less than 0.1 pH unit and the total elevation of $[SO_4^{2-}]$ over 5 d was ~0.10 mequiv·L^{-1}.

(ii) Haemolymph ionic status was assessed in *O. propinquus* subjected to similar experimental protocols (pH = 7.5, n = 12; pH = 4.0, n = 16) but sampled only on day 0 (200 μL) and day 5 (200 μL). On day 5, an additional 200 μL was drawn for lactate analysis in five to seven animals from both groups, as well as from a previously unsampled control group.

(iii) In order to follow the temporal development of ionic disturbances, the larger *O. rusticus* were subjected to the same regimes (pH = 7.5, n = 6; pH = 4.0, n = 6) but sampled daily (200 μL) from day 0 through to day 5.

(iv) Unidirectional fluxes of Na^+ and Cl^- and net fluxes of Na^+, Cl^-, K^+, Ca^{2+}, SO_4^{2-} (in five or six animals of each group only), ammonia, titratable acidity, and acidic equivalents were measured on a daily basis in *O. propinquus* on day 0, followed by 5 experimental days either at pH = 7.5 (n = 26) or pH = 4.0 (n = 23). In 10 animals from each group, fluxes were also measured during 5 d of recovery at pH = 7.5. The schedule was arranged so that the day 1 flux represented the first 5 h of exposure to low pH, and the day 1 flux during recovery represented the first 5 h of return to pH = 7.5. The animals were held in the 60-L tanks at appropriate pH as in the previous series, except during the actual 5-h flux measurements. For these, the animals were placed individually in small, well-aerated plastic containers filled with water of the correct pH. The control series served as a check on the possible disturbing effects of the brief handling and air exposure (<5 s) involved in these transfers. The ratio of water to animals was 50:1. These conditions represented the best compromise between water pH control and analytical sensitivity on the basis of 10 preliminary experiments employing various times and volume ratios. Water pH typically rose by about 0.3 unit (at pH = 4.0) over the 5-h run. All water used in the flux experiments was taken from two freshly made batches, either pH = 7.5 or pH = 4.0; both were spiked with 0.185 kBq (5×10^{-3} μCi) $^{22}Na \cdot mL^{-1}$ and 0.092 kBq (2.5×10^{-3} μCi) $^{36}Cl \cdot mL^{-1}$ (NEN). Fluxes were calculated from changes of concentration of substances in the closed system over the 5-h period.

Analyses and Calculations

Haemolymph acid–base status was assessed by standard theory and Radiometer electrode methodology as described previously (McDonald et al. 1979; Wood and Randall 1981). The measurements were difficult because of the low sample volumes (50 μL) and the very rapid clotting (<30 s) which necessitated disassembly and cleaning of the pH electrode after

every measurement, and precluded direct P_{CO_2} measurements. Approximately 25 μL was used for direct pHa measurement in a Radiometer E5021 capillary electrode at 10°C, and exactly 20 μL was injected into a miniature Cameron (1979) chamber (volume = 1.1 mL) for measurement of total CO_2 (Ca_{CO_2}). Pa_{CO_2} (in torr; 1 torr = 133.32 Pa) and $[HCO_3^-]$ (incorporating CO_3^{2-} and carbamino-CO_2) were calculated indirectly via the Henderson–Hasselbalch equation as outlined by McDonald et al. (1979) using pK^1 and αCO_2 values from the nomograms of Truchot (1976) at the appropriate temperature and ionic strength.

The concentration of acidic equivalents (ΔH_h^+) added to the haemolymph over time ("haemolymph metabolic acid load") was calculated as

$$(1)\quad \Delta H^+_h = [HCO_3^-]_1 - [HCO_3^-]_2 - \beta\,(pH_1 - pH_2)$$

where β is the nonbicarbonate buffer capacity of the haemolymph in slykes (i.e. $-\Delta HCO_3^- \cdot \Delta pH^{-1}$; Wood and Randall 1981). In vitro tonometry of pooled, declotted haemolymph from three and four specimens of *O. propinquus*, using techniques described by McDonald et al. (1979), yielded values of 7.4 and 8.5 slykes, respectively. Wilkes et al. (1980) reported β values for 10 individual *O. rusticus* in the range 7–11 slykes. A β value of 8 slykes was therefore used in the present calculations.

Haemolymph (200 μL) was immediately fixed in 400 μL of ice-cold 8% $HClO_3$ for subsequent lactate determination by the lactic dehydrogenase/NADH method (Sigma 1977). We did not observe the end-point drift reported in this assay by Graham et al. (1983), so chelating agents were not employed. Haemolymph for other ions was stored at −20°C. Upon thawing, the sample was mechanically disrupted and centrifuged at 10 000 × *g* for 5 min to separate clots. $[Na^+]$, $[K^+]$ (Eel MkII), and $[Ca^{2+}]$ (Coleman 20) were detected by flame photometry, appropriate swamping being used to remove interference effects. $[Cl^-]$ was determined by coulometric titration (Radiometer CMT10).

Water from the flux experiments was analyzed immediately for titratable alkalinity by titration of air-equilibrated 10-mL samples to pH = 4.00 with 0.02 *N* HCl as described by McDonald and Wood (1981). The remainder of the sample was frozen for later determination of Na^+, K^+, and Ca^{2+} (as for haemolymph); Cl^- (by coulometric titration on a Buchler-Cotlove 4-2000 chloridometer); total ammonia (by a micromodification of the phenolhypochlorite method of Solorzano 1969); and in some cases SO_4^{2-} (by the turbidometric method of Jackson and McCandless 1978). Net flux rates of each substance were calculated as

$$(2)\quad J_{net} = \frac{([X]_i - [X]_f)\cdot V}{t\cdot W}$$

where *i* and *f* refer to initial and final concentrations (nanoequivalents per millilitre), *V* the volume of the system (millilitres), *t* the elapsed time (hours), and *W* the body weight (grams). Thus, net losses by the animal have a negative sign, and net gains a positive sign. By reversing the *i* and *f* terms, the net titratable acidity flux was calculated from the titratable alkalinities. The sum of the titratable acidity (J_{net}^{TA}) and ammonia (J_{net}^{Amm}) fluxes, signs considered, yielded the net flux of acidic equivalents ($J_{net}^{H^+}$) which derives from the original principles outlined by Maetz (1973). As McDonald and Wood (1981) pointed out, this method does not distinguish between ammonia movement in the NH_3 and NH_4^+ forms, nor between the net excretion of acidic equivalents and the net uptake of basic equivalents, or vice versa. Fortunately this does not matter in terms of net acid–base balance.

Since ^{36}Cl is a pure β-emitter, while ^{22}Na is a mixed γ- and β-emitter, the cpm of each in a sample for unidirectional flux determinations could be separated by difference after counting in a γ-counter and a scintillation counter, as described by Wood et al. (1984). Unidirectional influxes (J_{in}) of Na^+ and Cl^- were calculated as outlined by Maetz (1956):

$$(3)\quad J_{in} = \frac{(R_i - R_f)\cdot V}{SA\cdot t\cdot W}$$

where R_i and R_f are initial and final radioactivities (cpm per millilitre), SA the mean specific activity (cpm per nanoequivalent) over the flux period, and the other symbols as in eq. (2). Preliminary analysis by the logarithmic model of Kirschner (1970) gave virtually identical results. In those instances where calculated internal specific activity exceeded 5% of external specific activity, backflux correction was performed as described by Maetz (1956). Unidirectional effluxes (J_{out}) were calculated by the conservation equation

$$(4)\quad J_{out} = J_{net} - J_{in}.$$

Data have been expressed as means ± SE (*n*) unless otherwise stated. The significance ($p \leq 0.05$) of differences between means was assessed using Student's two-tailed *t*-test, using either a paired (within groups) or unpaired (between groups) design as appropriate. Differences ($p \leq 0.05$) in mortality between control and experimental groups were assessed by a χ^2-test, one-tailed.

Results

Exposure of *O. propinquus* to pH = 4.0 in soft water for 5 d resulted in 28% mortality (19/69), significantly higher than the control mortality of 11% (6/53). At least two instances of the latter were due to cannibalism. In those animals in which the acid exposure was continued for 12 d, mortality rose to 54% (7/13), significantly higher than 10% (1/10) in the control group. There was no mortality in *O. rusticus* during 5 d at pH = 4.0.

Haemolymph Acid–Base and Ionic Status

Acid exposure caused a progressive fall in haemolymph pHa in *O. propinquus* from ~7.8 to ~7.3 by days 4 and 5 (Fig. 1A). While the acidosis was accompanied by a rise in Pa_{CO_2} during the first 2 d (Fig. 1C), the major effect was a progressive decline in haemolymph $[HCO_3^-]$ from ~7 to ~2 mequiv·L^{-1} (Fig. 1B). At 12 d, pHa was the same as at 5 d, but $[HCO_3^-]$ had fallen to less than 1 mequiv·L^{-1}, while Pa_{CO_2} (~0.7 torr) had dropped to about 25% of the day 0 level. Sampling caused small but significant changes in all three factors in the control group (Fig. 1), but these contributed marginally, if at all, to the effects seen in the experimental group. On a pH–HCO_3^- diagram (Fig. 2A), it was clear that the predominant effect until the end of day 4 was a metabolic acidosis with HCO_3^- loss at more or less constant Pa_{CO_2}. Thereafter, the compensation which stabilized pHa on days 5–12 was respiratory, i.e. a very large reduction in Pa_{CO_2}. The calculated "metabolic acid load" (ΔH_h^+) in the haemolymph was almost 4 mequiv·L^{-1} after only 24 h of acid exposure, reached

TABLE 1. Concentrations of major electrolytes (mequiv·L^{-1}, means ± 1 SE (n)) in arterial haemolymph of *O. propinquus* before (day 0) and after (day 5) exposure to either pH = 4.0 (experimental) or pH = 7.5 (control) for 5 d. *Significantly different ($p < 0.05$) from corresponding day 0 value.

	Day 0		Day 5	
	Control	Experimental	Control	Experimental
Na^+	191.9±9.2 (11)	192.9±8.3 (11)	186.7±6.5 (11)	147.2±11.2 (11)*
Cl^-	182.7±10.2 (12)	177.9±12.1 (11)	175.6±10.4 (12)	145.7±15.3 (11)*
K^+	3.65±0.62 (11)	3.45±0.34 (16)	3.22±0.49 (11)*	2.86±0.27 (16)*
Ca^{2+}	20.40±1.53 (12)	19.95±1.71 (16)	22.91±2.06 (12)	27.28±1.41 (16)*

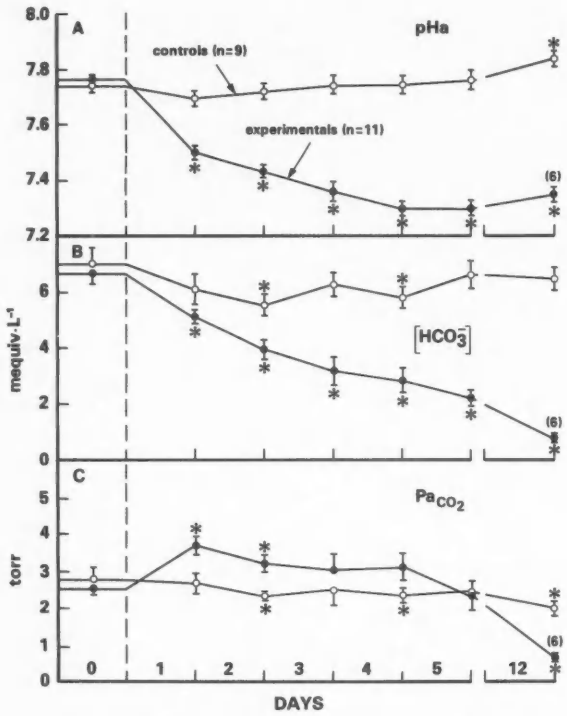

FIG. 1. Changes in (A) pH, (B) bicarbonate concentration (incorporating CO_3^{2-} and carbamino-CO_2), and (C) the partial pressure of CO_2 in the arterial haemolymph of *O. propinquus* during exposure to pH = 4.0 (1 torr = 133.332 Pa). The experimental animals were transferred from pH = 7.5 to pH = 4.0 after day 0. The control animals were kept at pH = 7.5 throughout. Data are means ± 1 SE. Asterisks indicate points significantly different from day 0 value for each group.

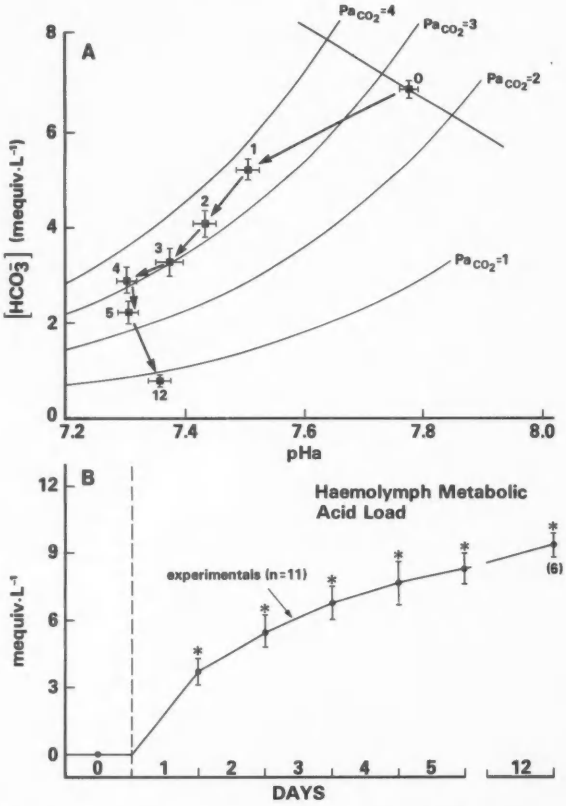

FIG. 2. (A) Changes in the acid–base status of arterial haemolymph in *O. propinquus* during 12 d of exposure to pH = 4.0, displayed on a pH–HCO_3 diagram. HCO_3 incorporates CO_3^{2-} and carbamino-CO_2. The Pa_{CO_2} isopleths are in torr (1 torr = 133.332 Pa). Data are means ± 1 SE; n as in part B. The diagonal line plotted through the day 0 value is a typical nonbicarbonate buffer line for haemolymph with a slope (β) of 8 slykes. (B) Calculated haemolymph metabolic acid load (ΔH_h^+) in *O. propinquus* during 12 d of exposure to pH = 4.0. Data are means ± 1 SE. Asterisks indicate points significantly different from the day 0 value which by definition is 0.

8 mequiv·L^{-1} after 5 d, and ~9.5 mequiv·L^{-1} by 12 d. This accumulation of acidic equivalents was not associated with a buildup of lactate, which remained very low in the haemolymph in both control (0.89 ± 0.10(7) mequiv·L^{-1}) and experimental animals (0.57 ± 0.16(5) mequiv·L^{-1}) after 5 d of exposure (Table 1) versus 0.68 ± 0.19(6) mequiv·L^{-1} in previously unsampled control animals. None of these values differed significantly ($p > 0.05$).

Environmental acidity also affected haemolymph ionic status in *O. propinquus* (Table 1). After 5 d, haemolymph [Na^+] and [Cl^-] had both fallen significantly; the decline in [Na^+] (~46 mequiv·L^{-1}) was significantly greater than that in Cl^- (~32 mequiv·L^{-1}). These ions did not change in the control animals kept at pH = 7.5. [K^+], which was present in much lower concentration in the blood, also fell significantly but this is of doubtful importance, as similar changes occurred

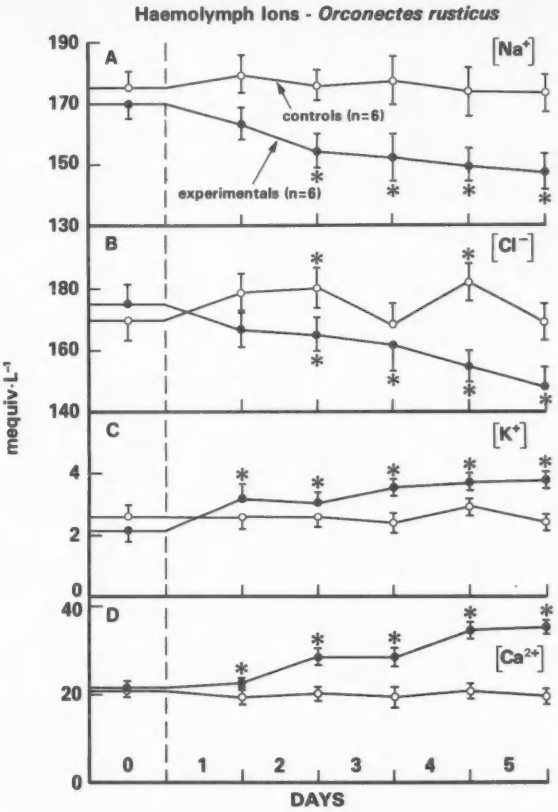

FIG. 3. Changes in (A) Na^+, (B) Cl^-, (C) K^+, and (D) Ca^{2+} concentrations in the arterial haemolymph of *O. rusticus* during exposure to pH = 4.0. The experimental animals were transferred from pH = 7.5 to pH = 4.0 after day 0. The control animals were kept at pH = 7.5 throughout. Data are means ± 1 SE. Asterisks indicate points significantly different from day 0 value for each group.

in the controls. On the other hand, haemolymph $[Ca^{2+}]$ increased significantly, while there was no change in the controls.

Haemolymph ions were measured on a daily basis in *O. rusticus* in an attempt to discern temporal patterns (Fig. 3A). However, the response appeared somewhat different than in *O. propinquus* because $[Na^+]$ and $[Cl^-]$ fell progressively to a much lesser extent and by equivalent amounts (~25 mequiv·L^{-1}) over 5 d. Furthermore, haemolymph $[K^+]$, which had decreased in *O. propinquus* (Table 1), almost doubled in *O. rusticus* (Fig. 3C). $[Ca^{2+}]$ rose progressively (Fig. 3D); the overall increase (~15 mequiv·L^{-1}) was about twice as large as that in *O. propinquus* (~7 mequiv·L^{-1}). Ion levels in the controls were relatively stable, the only apparent complication due to sampling being a small elevation of haemolymph $[Cl^-]$ on days 2 and 4 (Fig. 3B).

Ionic and Acidic Equivalent Exchanges with the Environment

The flux experiments were performed to see whether ionic and acid–base exchanges with the environmental water explained the observed haemolymph changes in *O. propinquus*. For the sake of simplicity, the data from those crayfish which

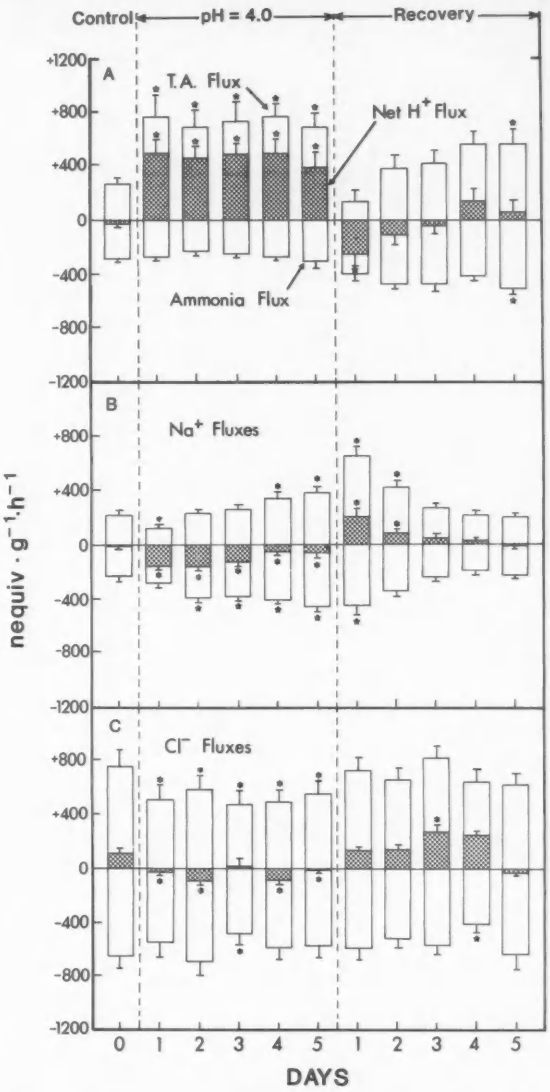

FIG. 4. Flux components in *O. propinquus* under control conditions at pH = 7.5, during 5 d of exposure to pH = 4.0, and during 5 d of recovery at pH = 7.5. (A) TA flux = titratable acidity flux (J_{net}^{TA}, upward bars), ammonia flux (J_{net}^{Amm}, downward bars), and the arithmetic sum of the two, the net acidic equivalent flux ($J_{net}^{H^+}$, stippled bars). (B) Unidirectional Na^+ influx ($J_{in}^{Na^+}$, upward bars), efflux ($J_{out}^{Na^+}$, downward bars), and net flux ($J_{net}^{Na^+}$, stippled bars). (C) Unidirectional Cl^- influx ($J_{in}^{Cl^-}$, upward bars), efflux ($J_{out}^{Cl^-}$, downward bars), and net flux ($J_{net}^{Cl^-}$, stippled bars). Data are means ± 1 SE; n = 23 on days 0–5 and n = 10 on days 1–5 recovery. Asterisks indicate points significantly different from the day 0 value.

were followed for 5 d of recovery after acid exposure have been pooled with those where the experiments were terminated after 5 d of exposure. In both control and experimental groups, there were relatively slight differences in the data between the two treatments, and none of these were significant on either day 0 or day 5. Furthermore, for all fluxes with the exception of K^+

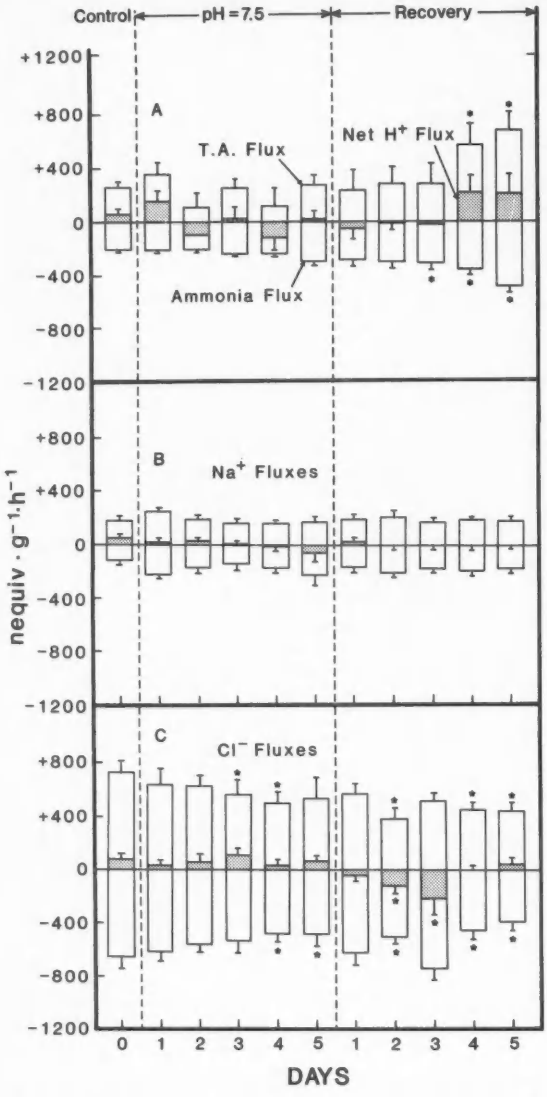

FIG. 5. Flux components in *O. propinquus* under maintained control conditions at pH = 7.5 in a regime otherwise duplicating the experimental regime of Fig. 4; $n = 26$ on days 0–5 and $n = 10$ on days 1–5 recovery. Other details as in Fig. 4.

(Fig. 6A versus 7A), the day 0 values were the same in the control and experimental crayfish.

On day 0 at pH = 7.5, crayfish were in net acid–base balance with the environment, the total ammonia excretion ($J_{net}^{Amm} \cong -250$ nequiv·g^{-1}·h^{-1}) just balancing the titratable acidity uptake ($J_{net}^{TA} \cong +250$ nequiv·g^{-1}·h^{-1}), so that $J_{net}^{H^+}$ was not significantly different from zero (Fig. 4A). The control experiment at pH = 7.5 (Fig. 5A) demonstrated that these exchanges were relatively stable over time except for increases in J_{net}^{TA} and J_{net}^{Amm} during the final 2–3 d of recovery. During 5 d at pH = 4.0, J_{net}^{Amm} remained unchanged, while J_{net}^{TA} increased two- to three-fold, resulting in highly positive values of $J_{net}^{H^+}$ ($\sim +450$ nequiv·g^{-1}·h^{-1}) throughout the exposure period (Fig. 4A). Upon return to pH = 7.5, there was an immediate drop in $J_{net}^{H^+}$ to below the day 0 level. J_{net}^{Amm} was again unchanged, so $J_{net}^{H^+}$ now became significantly negative. On subsequent days of recovery, the fluxes returned to day 0 levels. Therefore the large load of acidic equivalents taken up during 5 d of acid exposure did not appear to be fully excreted during recovery. However, the data must be interpreted with caution, as the actual flux measurements covered only 5 h of each 24-h period. The increases in both J_{net}^{TA} and J_{net}^{Amm} on the last day of recovery (Fig. 4A) can probably be related to the similar effects seen in the control group (Fig. 5A).

Unidirectional influxes and effluxes of Na^+ were both ~200 nequiv·g^{-1}·h^{-1}, resulting in negligible $J_{net}^{Na^+}$ under control conditions (Fig. 4B). The fluxes remained entirely stable during the 11-d control experiment at pH = 7.5 (Fig. 5B). Acid exposure caused an immediate net loss of Na^+ (~ -160 nequiv·g^{-1}·h^{-1}) due to a 50% inhibition of $J_{in}^{Na^+}$ at unchanged $J_{out}^{Na^+}$ (Fig. 4B). However, by day 2, $J_{in}^{Na^+}$ had recovered, and by day 4 and 5 had increased above the control level. In contrast, $J_{out}^{Na^+}$ gradually increased with time. The net effect was a moderation of Na^+ loss after day 2, but $J_{net}^{Na^+}$ still remained significantly depressed on day 5. Immediately upon return to pH = 7.5, $J_{in}^{Na^+}$ increased further to approximately threefold the original day 0 level. As $J_{out}^{Na^+}$ remained unchanged, $J_{net}^{Na^+}$ became highly positive ($\sim +210$ nequiv·g^{-1}·h^{-1}). During the ensuing 4 d of recovery, influx, efflux, and net flux components all gradually returned to their original levels. The total Na^+ losses during 5 d of acid exposure were approximately regained during 5 d of recovery.

At pH = 7.5, unidirectional fluxes of Cl^- (~700 nequiv·g^{-1}·h^{-1}) were about threefold greater than those of Na^+, and a slightly positive net balance occurred ($\sim +80$ nequiv·g^{-1}·h^{-1}; Fig. 4C). The control experiment revealed two complicating features of the protocol (Fig. 5C). Firstly, both $J_{in}^{Cl^-}$ and $J_{out}^{Cl^-}$ slowly declined by approximately 40% over the 11-d experiment, an effect which first became significant on day 3. Secondly, the slightly positive $J_{net}^{Cl^-}$ which persisted through the first 5 d became significantly negative by days 7 and 8, i.e. the second and third days of the recovery period. Despite these complications, it is clear that exposure to pH = 4.0 significantly reduced $J_{net}^{Cl^-}$, generally to negative values, while return to pH = 7.5 permitted full recovery of $J_{net}^{Cl^-}$ (Fig. 4C). On balance, it appeared that more Cl^- was gained during 5 d of recovery than was lost during 5 d of acid exposure. By comparison with the control experiment, these effects on $J_{net}^{Cl^-}$ appeared mainly due to a moderate (~30%) inhibition of $J_{in}^{Cl^-}$ during pH = 4.0 exposure and a stimulation of $J_{in}^{Cl^-}$ upon return to pH = 7.5, with minimal changes occurring in $J_{out}^{Cl^-}$.

On day 0, $J_{net}^{K^+}$ was positive ($\sim +80$ nequiv·g^{-1}·h^{-1}) in the experimental group (Fig. 6A), but approximately zero in the control animals (Fig. 7A); the reason for this difference is unknown. Nevertheless, the control experiment demonstrated stability of K^+ balance over the 11-d period (Fig. 7A). In contrast, $J_{net}^{K^+}$ became significantly negative (~ -80 nequiv·g^{-1}·h^{-1}) during 5 d at pH = 4.0 in the experimental group, an effect which persisted during the first 5 h of recovery at pH = 7.5 (Fig. 6A). By day 2, $J_{net}^{K^+}$ had returned to the day 0 level, but the overall K^+ loss during acid exposure was not completely restored during 5 d of recovery.

Rather surprisingly, Ca^{2+} balance was only moderately affected by environmental acidity. $J_{net}^{Ca^{2+}}$, which was close to zero

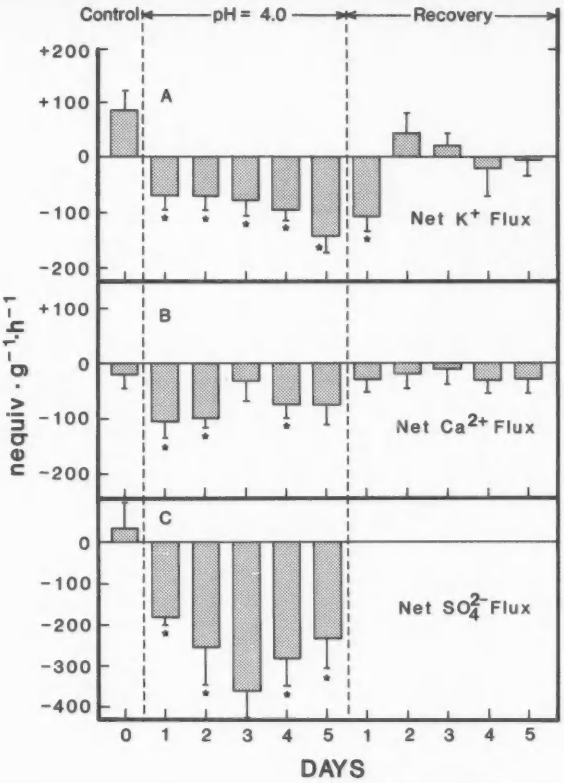

FIG. 6. Net fluxes of (A) K^+, (B) Ca^{2+}, and (C) SO_4^{2-} in *O. propinquus* under control conditions at pH = 7.5, during 5 d of exposure to pH = 4.0, and during 5 d of recovery at pH = 7.5. Data are means ± 1 SE; n = 23 on days 0–5 for K^+ and Ca^{2+}, n = 6 for SO_4^{2-}, and n = 10 on days 1–5 recovery. Asterisks indicate points significantly different from the day 0 values.

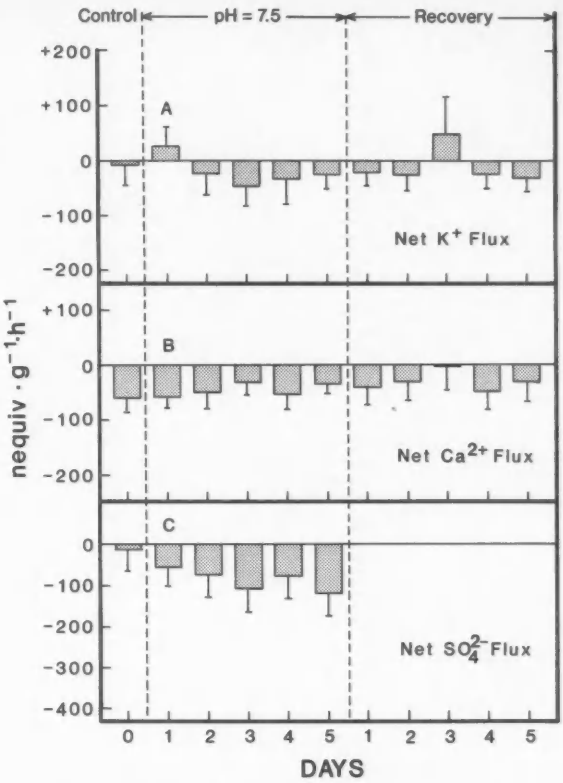

FIG. 7. Net fluxes in *O. propinquus* under maintained control conditions at pH = 7.5 in a regime otherwise duplicating the experimental regime of Fig. 6; n = 26 on days 0–5 for K^+ and Ca^{2+}, n = 5 for SO_4^{2-}, and n = 10 on days 1–5 recovery. Other details as in Fig. 6.

at pH = 7.5, became significantly negative on several days of the acid exposure, averaging ~−60 nequiv·g^{-1}·h^{-1} over the 5-d period (Fig. 6B). During recovery, $J_{net}^{Ca^{2+}}$ returned to the day 0 level. Ca^{2+} balance remained stable in the control animals throughout the experiment.

SO_4^{2-} fluxes were measured in only five or six animals in each group, and no data were obtained from the crayfish followed during the recovery period. At pH = 7.5, $J_{net}^{SO_4^{2-}}$ was close to zero, and there were no significant changes in the control animals (Fig. 6C, 7C). $J_{net}^{SO_4^{2-}}$ became significantly negative (~−250 nequiv·g^{-1}·h^{-1}) throughout the 5 d of acid exposure (Fig. 7C).

Discussion

Control Values

Although haemolymph ionic and acid–base status has not been previously studied in *O. propinquus*, our data for both this species and *O. rusticus* in soft water were generally similar to those reported for *O. rusticus* in hard water (Wilkes and McMahon 1982). We did not see the pronounced metabolic alkalosis and depressed haemolymph Cl^- levels reported for *Procambarus clarki* acclimated to decarbonated water at neutral pH (Morgan and McMahon 1982), perhaps because our water ion levels (especially Na^+ and Ca^{2+}) more closely duplicated those of natural low [HCO_3^-] soft water. Repetitive haemolymph sampling in control animals at neutral pH caused some disturbance of blood acid–base (Fig. 1) and ionic levels (Table 1; Fig. 3) as observed by others (e.g. Truchot 1975; McMahon et al. 1978; Morgan and McMahon 1982; McMahon and Morgan 1983), but these were generally small relative to the experimental effects of acid exposure.

These are the first data on acidic equivalent fluxes in crayfish and on unidirectional exchanges of Na^+ and Cl^- in softwater-acclimated crayfish. Previous studies were relatively short-term single isotope determinations in hard water or abnormal "experimental media," often on ionically depleted animals. Nevertheless, our data on *O. propinquus* are in broad agreement with previous studies on other genera (Shaw 1959, 1960c; Bryan 1960; Kirschner et al. 1973; Ehrenfeld 1974) in showing unidirectional Na^+ fluxes of ~200 nequiv·g^{-1}·h^{-1} and unidirectional Cl^- fluxes approximately threefold greater (Fig. 4, 5). These earlier studies characterized Na^+ and Cl^- uptakes as active, independent, electroneutral exchanges occurring at the gills. Whereas part of Na^+ uptake may be exchanged for acidic equivalents (H^+, NH_4^+) and part of Cl^- uptake for basic equivalents (HCO_3^-, OH^-), a large portion of J_{in} and J_{out} appears to reflect exchange diffusion for both Na^+ and Cl^-.

Our animals were in approximate net Na^+, Cl^-, Ca^{2+}, K^+,

SO_4^{2-}, and acidic equivalent balance at neutral pH (Fig. 4, 5, 6, 7), indicating that resting conditions and complete acclimation to soft water were achieved. Flux rates for most substances were stable under control conditions during the 11-d experiment (Fig. 5, 7), indicating that the experimental protocol itself was not particularly stressful. However, the disturbances in J_{net}^{TA} and J_{net}^{Amm} during the last 3 d (Fig. 5A) may have reflected some physiological deterioration, for by this point the animals had been starved 15–18 d. This may also have caused the gradual fall in unidirectional Cl^- exchanges and the negative J_{net}^{Cl} towards the end of the experiment (Fig. 5C). Alternately it is possible that the slow decline in J_{in}^{Cl} and J_{out}^{Cl} (but *not* the fall in J_{net}^{Cl}) was an experimental artifact. The error in Cl^- flux measurements was greater than in Na^+, for the ^{36}Cl cpm were obtained by subtraction. This, combined with the threefold greater turnover of Cl^-, may have incorporated a small but progressively increasing error in the backflux correction (cf. Maetz 1956) which would have reduced both J_{in}^{Cl} and J_{out}^{Cl} to the same absolute extent. Nevertheless, experimental effects of acid exposure (Fig. 5C) were still discernable.

Influence of Acid Exposure

Our study is the first to combine measures of internal acid–base and ionic status with determinations of the relevant exchanges with the environmental water in crayfish. As such, it allows quantitative analysis of the cause of disturbances seen during acid stress (Table 2), at least within the limitations of intermittent sampling of haemolymph and flux parameters.

The predominant haemolymph response to environmental acidity (pH = 4.0) in softwater-acclimated *O. propinquus* was a large metabolic acidosis (Fig. 1, 2). Similar effects have been seen in *O. rusticus* and *P. clarki* acclimated and tested in hard water at pH = 3.8 (Morgan and McMahon 1982; McMahon and Morgan 1983). In our study, the metabolic acidosis was clearly caused by the massive influx of acidic equivalents (or efflux of basic equivalents, i.e. positive $J_{net}^{H^+}$) from the environment (Fig. 4A), for there was no evidence of lactic acid production. Thus, there was probably no disturbance of O_2 delivery to the tissues. The elevated $J_{net}^{H^+}$ was virtually constant over the 5-d exposure (Fig. 4A), yet the accumulation of acidic equivalents in the haemolymph (i.e. ΔH_b^+) progressively slowed (Fig. 2B). Therefore, most of the acidic equivalent load was quickly moved out of the extracellular fluid volume (ECFV) and buffered in either the intracellular compartment (ICFV) or exoskeleton. Indeed, assuming an ECFV of 0.28 $mL \cdot g^{-1}$ (Kerley and Pritchard 1967), only about 4% of the total load was buffered in the ECFV at the end of 5 d (Table 2). The carapace in crustaceans contains an immense store of basic equivalents, mainly as $CaCO_3$ (Cameron and Wood 1985), so this was likely a major site of buffering. The elevated haemolymph Ca^{2+} levels (Fig. 3; Table 1) and net Ca^{2+} losses to the environment (Fig. 6B; Table 2) support this idea. We suggest that the intracellular compartment of muscle was another important buffer site, for acidic equivalent entry into muscle cells is generally associated with K^+ efflux (Ladé and Brown 1963) as observed here (Fig. 6A; Table 2). It is also possible that some of the observed $J_{net}^{H^+}$ occurred directly at the carapace–water interface, and never entered the haemolymph via the gills. The fact that $J_{net}^{H^+}$ (Fig. 3A) and $J_{net}^{Ca^{2+}}$ (Fig. 6B) returned to normal during recovery before apparent excretion of the total acidic equivalent load (Fig. 4A) suggests that some permanent demineralization of the exoskeleton occurred. This agrees with findings that postmoult calcification is inhibited by low pH in *O. virilis* in soft water (Malley 1980), and that carapace rigidity and Ca^{2+} content are reduced under acidification stress in the wild (France 1983).

TABLE 2. Total net fluxes (nequiv $\cdot g^{-1}$) with the environmental water over 5 d of acid exposure in *O. propinquus*, partitioned between the extracellular fluid volume (ECFV) and intracellular fluid volume (ICFV)/exoskeleton compartments.

	Total	ECFV[a]	ICFV/exoskeleton
H^+	+54 800	+2 300	+52 500
Na^+	−13 200	−11 300	−1 900
Cl^-	−5 300	−7 300	+2 000
K^+	−10 900	0	−10 900
Ca^{2+}	−9 100	+1 400	−10 500
Net charge[b]	+26 900	−300	+27 200

[a]Assuming ECFV = 28% body weight (Kerley and Pritchard 1967).
[b]Net charge = $(H^+ + Na^+ + K^+ + Ca^{2+}) - Cl^-$.

The elevation of Pa_{CO_2} during the first 2 d (Fig. 1C) also contributed to haemolymph acidosis and could have resulted from hypoventilation or a thickened diffusion barrier at the gills. Applying the analysis of Wood et al. (1977), increased Pa_{CO_2} accounted for 30–40% of the total pHa depression on days 1 and 2 and was negligible thereafter (Fig. 2A). The very low Pa_{CO_2} on day 12 served to reverse the metabolic pHa depression by ~25%, possibly a last ditch attempt to stave off fatal acidosis by hyperventilation. Interestingly, while *O. rusticus* showed a similar Pa_{CO_2} elevation, *P. clarki* exhibited decreased Pa_{CO_2} throughout 4 d of acid exposure (Morgan and McMahon 1982; McMahon and Morgan 1983).

While haemolymph electrolytes were also disturbed by low pH (Fig. 3; Table 1), the Na^+ and Cl^- depressions (~20%) were not as serious as the acid–base effects (70% loss of HCO_3^-, tripling of free H^+ concentration; Fig. 1). At least qualitatively, this was similar to the situation in *O. rusticus* and *P. clarki* in hard water (Morgan and McMahon 1982; McMahon and Morgan 1983). Losses of Na^+ and Cl^- from the haemolymph were almost entirely explained by losses to the environment, and net Na^+ and Cl^- fluxes with the ICFV or carapace were small (Table 2). In contrast, net Ca^{2+} and K^+ losses to the water (Fig. 6A, 6B) occurred entirely from outside the ECFV (Table 2), likely from the carapace and muscle compartments, respectively.

The unidirectional Na^+ and Cl^- flux measurements showed that net losses were initially due to modest inhibitions of $J_{in}^{Na^+}$ (by ~50%; Fig. 4B) and $J_{in}^{Cl^-}$ (by ~30%; Fig. 4C), while efflux rates were unaffected. We are aware of no previous work on low pH effects on Cl^- exchanges in freshwater crustaceans. However, the one previous study on Na^+ balance at low pH in crayfish (Shaw 1960b) showed similar effects (inhibited $J_{in}^{Na^+}$, unchanged $J_{out}^{Na^+}$). At pH = 4.0 in our experiments, H^+ ions were available in the external medium at half the concentration of Na^+ ions, so that 50% inhibition of $J_{in}^{Na^+}$, as well as some of the increase in $J_{net}^{H^+}$, could have resulted from H^+ versus Na^+ competition for a common carrier. The 30% inhibition of $J_{in}^{Cl^-}$ is more difficult to explain, but has been commonly observed in fish and amphibians (Wood and McDonald 1982; McDonald 1983a); it could reflect conformational changes in the carrier and/or the reduction of internal HCO_3^- levels (Fig. 1B) impeding Cl^-/HCO_3^- exchange. During continued acid exposure,

Cl^- fluxes remained stable at the day 1 level (Fig. 4C) but both $J_{in}^{Na^+}$ and $J_{out}^{Na^+}$ progressively increased (Fig. 4B). This could be explained by an increasing exchange diffusion component rather than recovery of Na^+ versus acidic equivalent exchange because $J_{out}^{Na^+}$ remained elevated upon return to neutral pH, whereas $J_{in}^{Na^+}$ showed a further immediate increase. The latter would represent restoration of Na^+ versus acidic equivalent exchange which in turn would restore positive $J_{net}^{Na^+}$, while the exchange diffusion component gradually declined over the 5-d recovery period.

Comparison with Fish

While the responses of *O. propinquus* to low pH were superficially similar to those of fish, their detailed nature was very different. In the gills of the rainbow trout (*Salmo gairdneri*), $J_{net}^{H^+}$ is constrained by the difference between strong cation (mainly Na^+) and strong anion (mainly Cl^-) fluxes (McDonald 1983b; Wood et al. 1984) as predicted by the strong ion difference concept (Stewart 1978) and the demands of electroneutrality. During acid stress in hard water (high $[Ca^{2+}]$) the gill epithelium becomes more permeable to Na^+ than to Cl^-, so positive $J_{net}^{H^+}$, acidosis, and Na^+ loss in excess of Cl^- loss occur (McDonald et al. 1980; McDonald and Wood 1981; Wood and McDonald 1982; McDonald 1983a, 1983b; McDonald et al. 1983). In contrast, in soft water (low $[Ca^{2+}]$), acid stress elevates Na^+ and Cl^- permeabilities by greater but approximately equal amounts, so $J_{net}^{H^+}$ is negligible, acidosis does not occur, but large equimolar losses of Na^+ and Cl^- result in rapid mortality. Thus, the response of the crayfish in *soft* water (highly positive $J_{net}^{H^+}$, severe acidosis, relatively small Na^+ loss considerably in excess of Cl^- loss) resembled that of the trout in *hard* water. Perhaps the higher Ca^{2+} levels in the blood and tissues of crayfish are responsible for a "hard-water type" response in soft water.

While the highly positive $J_{net}^{H^+}$ was correlated with a loss of strong cations (Na^+, K^+, and Ca^{2+}), far in excess of strong anion (Cl^-) as predicted by theory, a considerable charge imbalance remained, a deficit equal to ~50% of $J_{net}^{H^+}$ over 5 d (Table 2). This has not been seen in the trout studies. This deficit was associated entirely with the ICFV or exoskeleton, for good charge balance was achieved in the ECFV (Table 2). Although SO_4^{2-} is reportedly impermeant at the gills of crayfish (Shaw 1960a; Ehrenfeld 1974), we suspected that a direct entry of SO_4^{2-} into the carapace (in association with H^+ and effectively in exchange for CO_3^{2-}) might be responsible. While SO_4^{2-} fluxes were measured in only a few animals (Fig. 6C, 7C) and therefore the data not included in Table 2, this was clearly not the explanation. SO_4^{2-} was lost in significant amounts during acid exposure, which would exacerbate rather than reduce the charge imbalance of Table 2. The explanation remains unknown.

Further differences from fish occurred in the flux effects. In both rainbow trout (McDonald et al. 1983) and white sucker (*Catostomus commersoni*; Hōbe et al. 1984), pH ≅ 4.0 caused a greater immediate inhibition (60–95%) of both $J_{in}^{Na^+}$ and $J_{in}^{Cl^-}$, and very large diffusive increases in $J_{out}^{Na^+}$ and $J_{out}^{Cl^-}$. The latter were the major causes of ion loss during acute exposure. During continued acid stress, J_{out} values returned to control levels without recovery of J_{in} values, so the inhibition of active uptake was the major source of ion loss over the longer term in fish. This is very different from the crayfish where inhibitory effects on J_{in} components predominated throughout (Fig. 4B, 4C). While interpretation is clouded by the possibly changing role of exchange diffusion, this at least indicates that permeability of the gills to passive effluxes in crayfish is much more acid resistant than in fish. Furthermore, in fish, positive $J_{net}^{H^+}$ was attenuated during continued acid exposure (McDonald 1983b; McDonald et al. 1983; Hōbe et al. 1984). That this did not occur in crayfish (Fig. 4A) again suggests direct H^+ penetration of the carapace. Finally, unlike fish, acid exposure had no effect on J_{net}^{Amm} in *O. propinquus*, which suggests that Na^+ versus NH_4^+ exchange is of lesser importance in crayfish than in fish (cf. Wright and Wood 1985), in agreement with previous studies (Kirschner et al. 1973; Ehrenfeld 1974). While much further work is needed to understand the mechanisms behind acid stress responses in crayfish, it is already clear that extrapolation from fish data will be of only very limited help.

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Selected Articles on Fish Genetics: Introduction

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In recent years there has been increasing interest in the evolutionary biology of fishes and the impact of exploitation on the genetic status of existing species. This interest in fishes has also extended into other fields; for example, the experimental evidence collated from certain families has had a bearing on the way we view rates of evolution in other groups.

With this in mind, the *Journal's* Editorial Board put forward the idea of publishing a group of papers that would indicate not only areas of interest within the field of fish genetics, but those that might influence it in the future. The papers which follow therefore cover aspects of genetics that are generally seen in other more specialized journals; they do not include, for example, papers that are concerned with more "traditional" biochemical approaches to fish genetics, because the *Journal* already contains many excellent publications in this area.

The five papers that have been selected concern molecular characteristics through to behavioral aspects of genetics. In the first paper on fish antifreeze proteins and recent gene evolution (Scott, Fletcher, and Davies), we see data from HPLC protein separation and cDNA probes used in conjunction with evidence from paleoclimatology to present a phylogenetic and zoogeographic analysis of marine teleosts. The authors present the idea that trans-hemisphere migrations and timing of events such as the production of antifreeze proteins may in fact have been driven by Cenozoic cooling initiated in the Southern Hemisphere, an idea that is likely to cause some reaction. They also suggest a realignment of the nototheniids with the Gadiformes, a proposal that will no doubt increase our interest in resolving the existing differences that exist in the literature regarding the Acanthopterygii and Paracanthopterygii.

The second paper on protein folding (Foster) provides us with a distance constraint model for the prediction of tertiary structures, given only the primary structure of such globular proteins as lysozyme and pancreatic trypsin inhibitor. In this example, we see the potential for theoretical approaches to resolving the importance of functional attributes of groups of proteins given a general model for the low resolution of tertiary structures from a primary sequence.

The third paper on the evolutionary relationship of fish C type lactate dehydrogenase (LDH) to other vertebrate LDH isozymes (Rehse and Davidson) provides us with an alternative view to that commonly held, based on new and published amino acid composition and sequence data. The traditional model suggests that the ancestral LDH was an A type, whereas the evidence and arguments given in this paper suggest that the C type was ancestral.

The fourth paper presents data on functional aspects of enzymes in fishes and a model of the evolution of specialized and generalized enzymes (McGlade and Allen). The theoretical model is based on considerations of volume swept, binding time, and cost of production and looks at enzymes operating in dilute and concentrated environments and their ability to perform different functions or react with a variety of substrates.

The fifth paper concerns the problems associated with selection of growth and aggressive behavior in artificial selection programs using a game theoretic approach (Doyle and Talbot). The results provide an interesting illustration not only of this technique but also of the fact that rapid growth may not directly incur aggressive tendencies, but rather indirectly tameness. Given the interest in this type of approach, the inclusion of this paper looking at behavioral aspects of fish genetics is likely to produce increased research in the area.

It is hoped that the papers included will thus provide readers with a view of new developments both within fish genetics and in molecular biology that are likely to have some bearing on the future of fisheries research. (*J8692*)

Fish Antifreeze Proteins: Recent Gene Evolution[1]

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Scott, G. K., G. L. Fletcher, and P. L. Davies. 1986. Fish antifreeze proteins: recent gene evolution. Can. J. Fish. Aquat. Sci. 43: 1028–1034.

A variety of antifreeze proteins is produced by marine teleosts inhabiting polar regions to ensure protection from internal ice formation at subzero temperatures. Combining evidence from paleoclimatology, teleostian evolution, and studies of antifreeze gene organization, the case is made for Cenozoic cooling as the force driving antifreeze evolution in marine teleosts. The distribution of antifreeze types amongst teleost suborders, families, genera, and species correlates with Cenozoic glaciation in the Southern Hemisphere preceding that in the Northern Hemisphere by approximately 25 million yr. The genomic organization of antifreeze genes suggests recent and extensive amplification, an event compatible with their proposed stress-induced origin. Also, a realignment of the nototheniids with the Gadiformes based on antifreeze protein data is suggested.

Diverses protéines jouant le rôle d'antigel sont produites par des téléostéens marins qui habitent les régions polaires pour empêcher la formation de glace à l'intérieur de l'organisme à des températures inférieures à zéro. En combinant les indications fournies par la paléoclimatologie, l'évolution des téléostéens et les études sur l'organisation des gènes des protéines « antigel », on démontre que le refroidissement survenu au Cénozoïque a été le facteur qui a déclenché l'évolution des protéines « antigel » chez les téléostéens marins. La distribution des types de protéines « antigel » parmi les sous-ordres, les familles, les genres et les espèces de téléostéens se trouve en corrélation avec la glaciation du Cénozoïque dans l'hémisphère sud qui a précédé celle qui a eu lieu dans l'hémisphère nord il y a environ 25 millions d'années. L'organisation des génomes de gènes de protéines « antigel » laisse croire en une amplification récente et importante, un événement compatible avec leur origine prévue provoquée par le stress. À partir des données sur les protéines « antigel », on propose également un réalignement des nototheniidés avec les gadiformes.

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Need for Antifreeze in Marine Teleosts

Like most vertebrates, the teleosts, a group encompassing over 95% of all extant fish species, maintain a serum solute concentration approximately one-third that of seawater. In the presence of existing global climatic conditions and as a result of this disparity in solute concentrations, seawater temperatures at high latitudes typically run 1°C below the normal freezing point of teleost serum (−0.8°C). Some marine teleosts avoid this potentially lethal situation by migrating to warmer or deeper (100 m) waters in the winter (DeVries 1983). In deep water at temperatures below −0.8°C, fish appear to exist in a supercooled state made metastable by the absence of nucleating ice crystals (DeVries 1983). Other species, however, have adapted to life in ice-laden seawater by producing serum antifreezes which noncolligatively depress the serum freezing point to safe levels. The commitment to antifreeze production by marine teleosts is considerable, with serum levels reaching 10 mg/mL during winter conditions (DeVries 1971). On a mass basis these antifreezes, either peptides or glycopeptides, are from 200 to 300 times more effective in freezing point suppression than typical proteins like myoglobin (Duman and DeVries 1975; Fletcher 1977), but have no such differential effect on the melting point.

Phylogenetic Relationships and the Diversity of Fish Antifreezes

The proteins utilized by marine teleosts for antifreeze protection are remarkably diverse (Table 1). Each of these serum antifreezes comprises a set of closely related proteins, resolvable on HPLC into as few as 2 and as many as 11 components (Hew and Fletcher 1985). In Table 1, AFPI refers to the α-helical, alanine-rich (>60 mol%) peptides of the winter flounder (*Pseudopleuronectes americanus*) and shorthorn sculpin (*Myoxocephalus scorpius*). The antifreeze from the sea raven (*Hemitripterus americanus*) (AFPII) is relatively rich in cystine residues and β-structure, but is otherwise unremarkable. A third type of peptide antifreeze (AFPIII), found in the

[1]Contribution No. 610 of the Marine Sciences Research Laboratory, Memorial University of Newfoundland.

TABLE 1. Characteristics of four fish antifreeze types. Data was compiled from Hew (1981) and DeVries (1983). Amino acid compositions were determined on the mixture of antifreeze components present in the serum. Compositions of individual components do not differ significantly from those of the bulk antifreezes. Molecular weights of the antifreezes have been corrected. ND = not determined. The asterisk next to AFPI (winter flounder) distinguishes it from the structurally similar but probably genetically unrelated AFPI of the shorthorn sculpin.

	Polypeptide antifreeze				
	AFPI* winter flounder (mol%)	AFPI shorthorn sculpin (mol%)	AFPII sea raven (mol%)	AFPIII ocean pout (mol%)	Glycoprotein antifreeze: AFGP notothenids/cods (mol%)
Aspartic acid	11.5	6.4	10.7	12.0	
Threonine	9.9	6.4	7.9	11.4	33
Serine	3.3	1.6	8.2	9.7	
Proline	0	1.6	6.7	8.9	Present
Glutamic acid	1.6	3.2	9.1	12.4	
Glycine	0	1.6	8.1	6.4	
Alanine	62.8	60.8	14.4	11.2	66
Half-cystine	0	0	7.6	0.8	
Valine	0	0	1.2	11.0	
Methionine	0	2.4	5.4	1.2	
Isoleucine	0	1.6	1.7	6.8	
Leucine	5.8	4.0	6.2	4.7	
Tyrosine	0	0	1.2	1.3	
Phenylalanine	0	0	2.0	1.5	
Lysine	3.3	8.0	2.1	6.2	
Histidine	0	0	2.5	0	
Tryptophan	0	0	2.8	ND	
Arginine	1.6	2.4	2.3	0.5	
Hexosamine	0	0	0	0	Present
Mol. wt.	4000	3000–5000	10 000	6500	2600–33 000
Secondary structure	α-Helix	α-Helix	β-Structure		Expanded structure

ocean pout (*Macrozoarces americanus*), has even less bias in its amino acid composition and no dominant type of secondary structure. The last column describes the glycoprotein antifreezes (AFGP) of the notothenids and Gadidae which are made up of a repeating tripeptide unit $(-ala-ala-thr)_n$ with a disaccharide moiety attached to the threonine side chain. The distinction between antifreeze types is further emphasised by a comparison of their primary sequences (Hew and Fletcher 1985).

An attempt to correlate antifreeze type with the assumed phylogenetic relationships between fishes and their zoogeographical distribution leads to many anomalous situations. The four major antifreeze types characterized to date are distributed over seven families spanning at least five suborders (Fig. 1). Some closely related fishes inhabiting the same environment produce very different antifreezes, while others thought to be of different orders and inhabiting opposite polar waters produce essentially identical antifreezes. This puzzling state of affairs has been remarked upon (DeVries 1983), but not explained. In the following, we offer the hypothesis of climatically driven gene evolution to explain the distribution of antifreeze types in marine teleosts.

We suggest that the first antifreeze proteins of marine teleosts were established during the dramatic Cenozoic cooling events initiated approximately 30 million yr ago at the Eocene–Oligocene boundary (Kennet and Shackleton 1976; Keigwin 1980), and that some of them have evolved as recently as 2.5 million yr ago. We find the variety and geographical dispersion of antifreeze types to be in accord with results from paleoclimatology and the general pattern of teleostean evolution. In particular, the temporal development of Cenozoic cooling, where glaciation in Antarctica (Kerr 1984a) precedes Northern Hemisphere glaciation (Shackleton et al. 1984) by approximately 25 million yr, appears evident in the present zoogeographical distribution of antifreeze types among marine teleosts. In addition to this phylogenetic evidence, studies of the genomic organization of antifreeze genes are revealing amplified structures suggestive of recent evolution. We briefly summarize results from paleoclimatology and teleostean evolution that are pertinent to our treatment of antifreeze evolution driven by Cenozoic cooling.

Teleost Emergence

The teleosts, the most recently evolved group of fishes, originally appeared as rare forms in the Triassic period 200 million yr ago (Patterson 1964; Greenwood et al. 1966). Although the fossil record of the early teleosts is fragmented, it is presumed that they arose from holostean stock and had effectively replaced the older holosteans by the beginning of the Cretaceous about 130 million yr ago. By the Eocene, approximately 40 million yr ago, teleostean main faunas had achieved a definite morphological resemblance to the modern assemblage (Greenwood et al. 1966).

The teleostean emergence and radiation occurred in an environment where globally averaged temperatures were from at

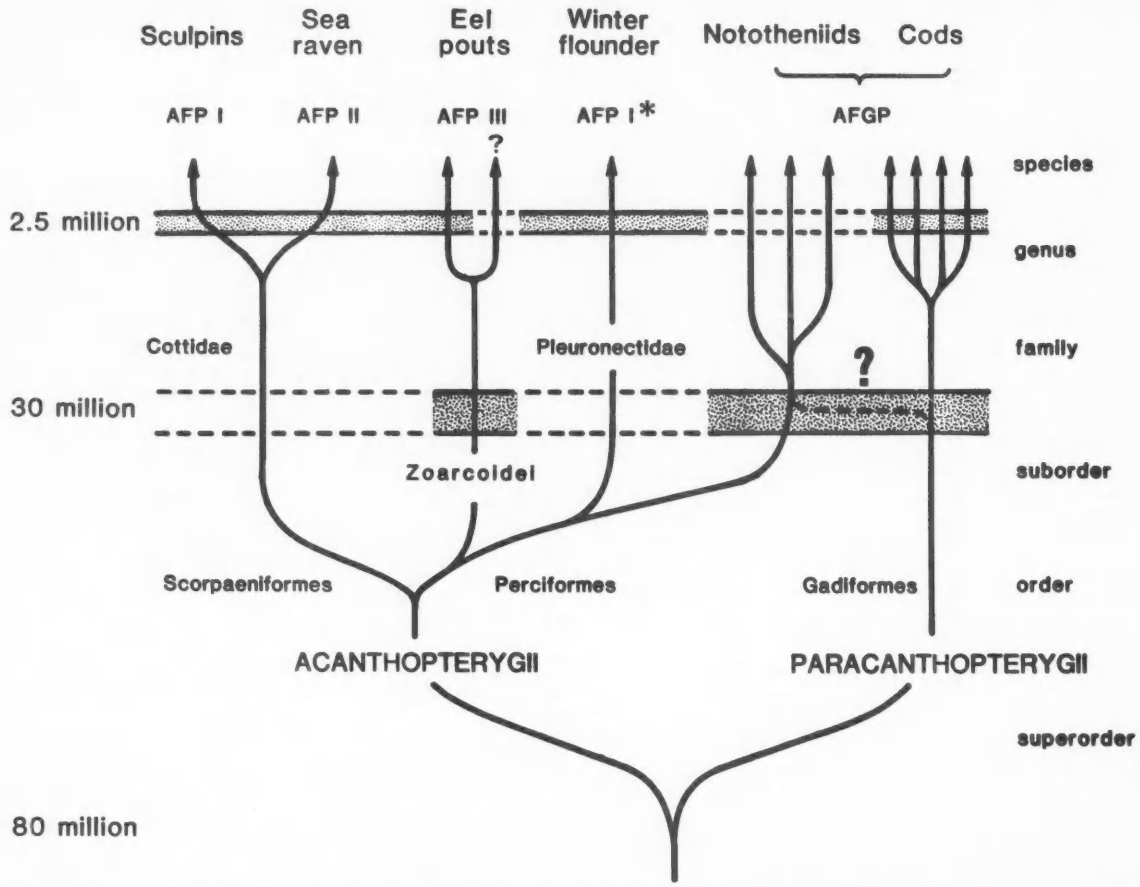


FIG. 1. Schematic outline showing the phylogenetic relationships of present day fish that have well-characterized antifreeze proteins (adapted from Greenwood et al. (1966) except for the placement of Zoarcoidei). The vertical time scale on the left in years is correlated on the right-hand side with progress towards present day speciation in the superorders Acanthopterygii and Paracanthopterygii. The broad band at 30 million yr indicates the onset of Antarctic glaciation. Stippled sections encompass lineages which developed antifreezes in this era. A similar band at 2.5 million yr indicates the onset of Arctic glaciation, where again stippled sections encompass lineages exposed to this climatic cooling. The nomenclature regarding antifreeze types is that used in Table 1. This scheme shows lineages which have been challenged to produce antifreeze in the Northern Hemisphere only, in the Southern Hemisphere only, and lineages which were challenged first in the Antarctic and again, after trans-hemisphere migration, in the Northern Hemisphere. Question marks indicate uncertainty about the antifreeze types in the Antarctic eelpout, and the time of divergence of the notothenilds and cods.

least 6°C to as much as 14°C higher than today (Kerr 1984b; Barron 1984). Significantly warmer were the polar regions where the temperature differential between poles and equator was no more than 26°C as compared with the 41°C difference existing today. This warm global climate, initiated at the close of the Permocarboniferous ice age 250 million yr ago, prevailed essentially uninterrupted for approximately 200 million yr before a gradual cooling trend became established at the close of the Cretaceous (Denton et al. 1980; Lewin 1984). Rapid intensification of this global cooling trend appears at the Eocene–Oligocene boundary (Kennet and Shackleton 1976; Keigwin 1980). Oxygen isotope ratios from marine sediments suggest the formation of Antarctic ice sheets as early as 30 million yr ago, and major ice accumulation there 16 million yr ago (Kerr 1984a; Denton et al. 1971). Evidence for Northern Hemisphere glaciation first appears in the North Atlantic only 2.5 million yr ago (Shackleton et al. 1984). Correlation of these glaciations with variations in the faunal record is presently an active area of research (Lewin 1984).

Impact of Glaciation: Phylogenetic Evidence

With teleostean emergence staged during warm climatic conditions, the advent of Cenozoic glaciation challenged teleosts for the first time with water temperatures below their serum freezing level. The modern teleosts had either established or were on the verge of establishing at least family integrity by the onset of Antarctic glaciation approximately 30 million yr ago, and genus or species integrity by the onset of Arctic glaciation 2.5 million yr ago (Greenwood et al. 1966). Thus, there have been at least two major cooling events subsequent to teleost suborder diversification (Fig. 1). Accordingly, the phyloge-

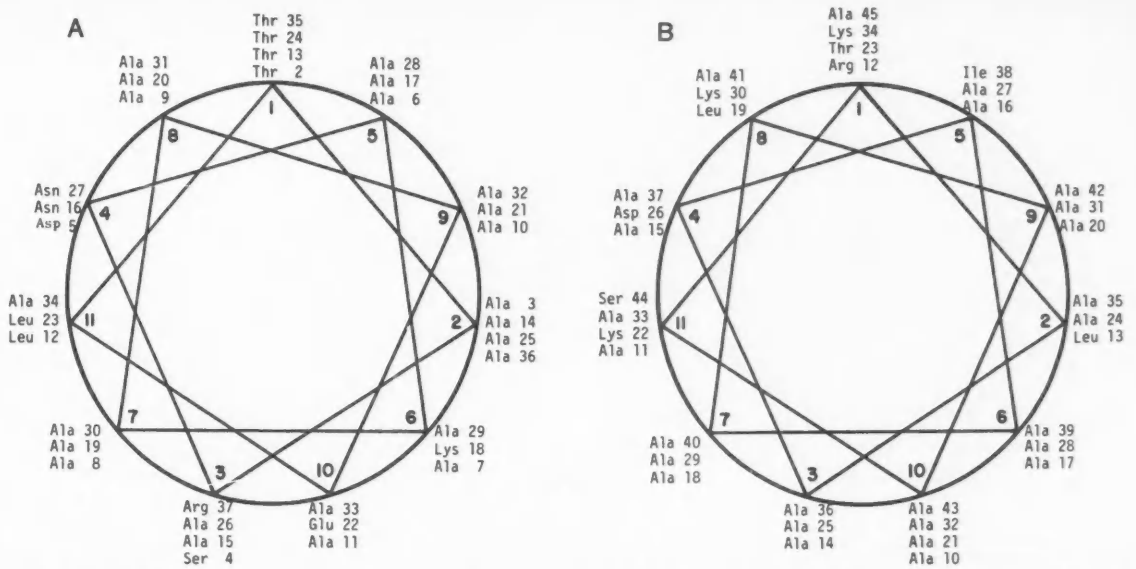


FIG. 2. Helical projections for (A) winter flounder and (B) shorthorn sculpin antifreezes based on an 11-residue repeat. (A) Residues 2–37 inclusive of the winter flounder antifreeze protein A component; (B) residues 10–45 inclusive of the shorthorn sculpin antifreeze protein component 8. Residues are numbered from the amino termini.

netic relationships and distribution of antifreeze-producing teleosts argue for at least two episodes of antifreeze evolution, one in the Southern Hemisphere and another in the Northern Hemisphere.

Southern Hemisphere

The impact of Antarctic glaciation is best discernible in the suborder Notothenioidei. With habitat strictly confined to the South polar region, at least three families from this suborder produce glycoprotein antifreezes which are essentially identical in size, sequence, and number of components (Duman and DeVries 1975; Fletcher 1977). The appearance of identical antifreezes across these different family lines is consistent with climatic stress driving their development slightly before or during family diversification 30–40 million yr ago.

One other antifreeze type has been described in an Antarctic fish (DeVries 1980). This is the peptide antifreeze of *Rhigophila dearborni*, an eelpout in the family Zoarcidae. Since this family is in a different suborder than the notothenioids, the distinction between their antifreeze types is consistent with separate evolution occurring after suborder divergence (Fig. 1). The Zoarcidae also have representatives in the Northern Hemisphere that produce antifreezes. Antifreezes from one of the northern species, *M. americanus*, have recently been characterized (Hew et al. 1984) and sequenced (Hew and Fletcher 1985). They are also peptide antifreezes, but a detailed comparison with the *R. dearborni* antifreezes awaits better characterization of the latter proteins. If their structures are similar, the suggestion would be that this antifreeze type evolved in the Southern Hemisphere during early Antarctic glaciation and has appeared in the north due to the trans-hemisphere migration of *M. americanus* antecedents. In support of this suggestion it should be noted that the southernmost section of the Americas is the area of highest Zoarcid generic endemism (Anderson 1984). Less probable is antifreeze evolution by the eelpouts in the Northern Hemisphere followed by migration to the Antarctic due to the increasing isolation of Antarctica by the strong circumpolar current. This current was initiated at the onset of Cenozoic cooling and was well established 2.5 million yr ago (Lewin 1984). The issue of trans-hemisphere migration emerges again in discussion of the cods.

Northern Hemisphere: The Cottids

Phylogenetic evidence for a more recent episode of antifreeze evolution is best illustrated by comparing the antifreezes from two cottids, the shorthorn sculpin and sea raven. Both cottids, members of the same family, bear a close physical resemblance to each other and have a habitat confined to the North Atlantic (Nelson 1984). However, they produce distinctly different antifreeze types (Table 1). The shorthorn sculpin antifreezes are alanine-rich α-helical peptides which lack cystine, while the sea raven antifreezes have a low α-helix content and depend on the oxidized form of their cystine residues for activity. The distinctive character of these two antifreeze types signifies their independent evolution. With Northern Hemisphere glaciation initiated only 2.5 million yr ago, it is recent enough to resolve the small evolutionary separation between sculpin and sea raven.

Convergent Evolution: The Alanine-Rich Antifreeze Proteins

What seems at first sight to be an anomaly in the pattern of antifreeze evolution in the Northern Hemisphere is the resemblance of the winter flounder antifreeze protein to that produced by the distantly related shorthorn sculpin (Table 1). Both proteins are alanine rich (>60 mol%) with a limited number of other amino acids present, and both are almost entirely α-helix. The striking structural feature of the flounder antifreezes is

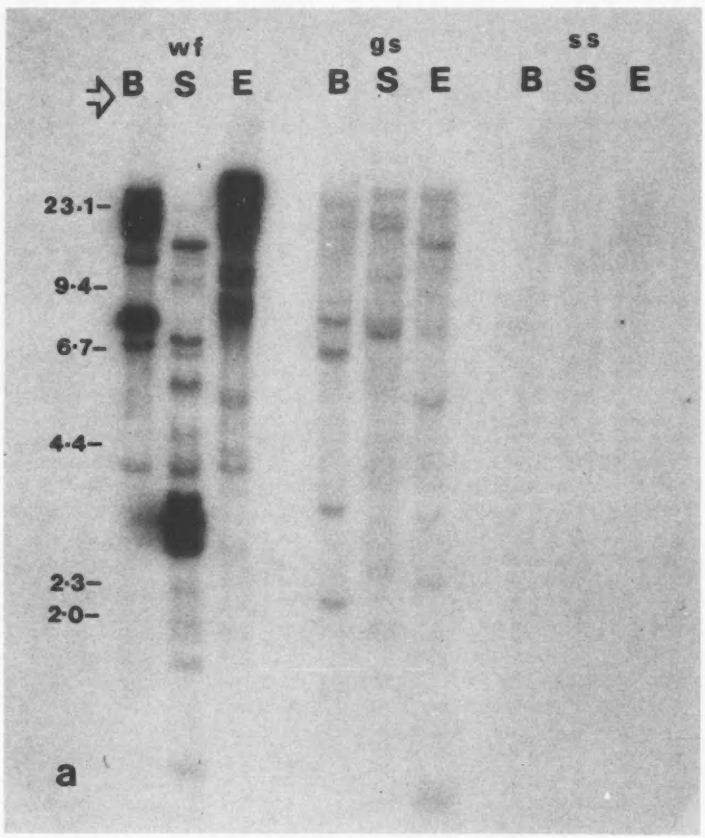
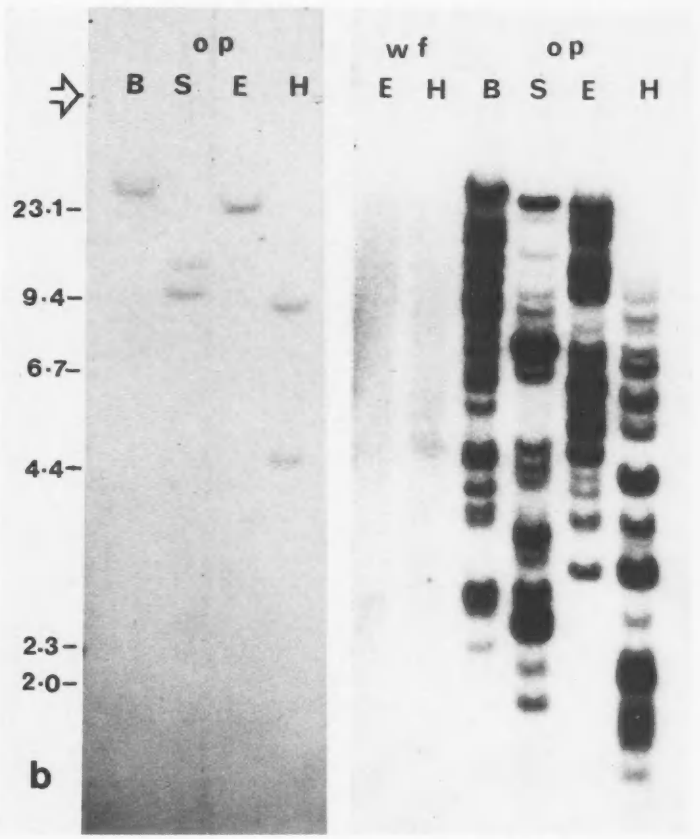


FIG. 3. Genomic Southern blots of fish DNAs. (a) Aliquots of winter flounder (wf) DNA (4 μg), g
Bam H1 (B), Sst I (S), or Eco R1 (E), electrophoresed on a 0.7% agarose gel, blotted onto nitroc
clone (Davies et al. 1984). The blot was washed at 64°C in 0.25 × SSC. Major hybridizing bands
lanes are derived from the tandemly organized antifreeze protein genes (Davies et al. 1984). DNA
of sample application. (b) Aliquots of ocean pout (op) DNA (15 μg) were digested with Bam H1 (B
the latter two enzymes. The blots were prepared as described above. The left-hand set of ocean pout D
other blot was probed with an ocean pout antifreeze protein cDNA clone. Both blots were washe



(4 μg), grey sole (gs) DNA (15 μg), and shorthorn sculpin (ss) DNA (15 μg) were digested with
to nitrocellulose, and probed with a nick-translated winter flounder antifreeze protein genomic
g bands at 7.8 kbp (Bam H1), 2.8 kbp (Sst 1), and >20 kbp (Eco R1) in the winter flounder
). DNA markers were derived from a Hind III digest of λ DNA. The arrow indicates the point
m H1 (B), Sst 1 (S), Eco R1 (E), and Hind III (H) and winter flounder (wf) DNA (15 μg) with
n pout DNAs were probed with a cDNA copy of a presumed single-copy ocean pout gene. The
re washed at 67°C in 0.15 × SSC.

their three 11-amino-acid repeats which begin with threonine and have aspartic acid or asparagine in the fourth position. When organized into an α-helix as shown by the helical projection in Fig. 2, these residues are clustered on one side. According to the model proposed by DeVries, this would be the side of the protein which hydrogen bonds to the ice lattice to inhibit crystal growth in the most favoured direction (DeVries 1983). When the antifreeze components from the flounder and sculpin are compared (Hew and Fletcher 1985) they show approximately 60% homology. When the C-terminal portion of the sculpin antifreeze is displayed as a helical diagram, it forms a perfect amphipathic α-helix with all the hydrophilic side chains projecting from one quadrant (Fig. 2). Thus the sculpin and flounder antifreezes have similar secondary structures, and, therefore, probably function in the same manner. However, using flounder AFP cDNA probes, we have been unable to detect any homology to sculpin liver RNA on Northern blots (P. L. Davies et al., unpubl.), or to sculpin genomic DNA on Southern blots (Fig. 3a) even under relaxed hybridization and washing conditions. We conclude from these observations that the flounder and sculpin progenitors utilized different genetic elements to evolve similar types of antifreeze.

The Cods

Worthy of comment on purely phylogenetic grounds is the appearance of glycopeptide antifreezes in Northern Hemisphere Gadiformes which are essentially identical to those of the nototheniids (Van Voorhies et al. 1978; Osuga and Feeney 1978; Hew et al. 1981). These antifreezes constitute a well-defined set of components in which the relative electrophoretic mobilities and band intensities of individual components are remarkably conserved between Gadiformes and nototheniids. Unlike the situation with shorthorn sculpin and winter flounder where the appearance of the same antifreeze type probably reflects convergent evolution of distinct genetic elements, perfect parallel evolution of a complex antifreeze type by two orders presumed well separated is difficult to accept. In their seminal work on the evolutionary relationships of the modern teleosts, Greenwood et al. (1966), although placing the Notothenioidei with the advanced Perciformes, and the Zoarcidae with the less advanced Gadiformes, commented on morphological variables making this classification ambiguous. Indeed, in the older literature the Notothenioidei were grouped with the Gadiformes on the basis of skeletal similarities (Boulenger 1901). Subsequently, using lactate dehydrogenase isoenzyme analysis to distinguish between higher and lower teleosts (Whitt 1969), the placement of Zoarcidae with the higher perciformes appears established (Nelson 1984; Shaklee and Whitt 1981). To our knowledge, no lactate dehydrogenase analysis has been performed on the nototheniids, although this analysis exists for the Gadiformes (Whitt 1969). We conjecture that lactate dehydrogenase analysis of nototheniids would indicate their alliance with the more primitive Gadiformes and support the argument that these two groups diverged approximately 30–40 million yr ago after their glycopeptide antifreeze development had been established. Assuming this is the case, the inference is that the Gadidae, as was suggested for the Zoarcidae, radiated northward from Antarctica with their antifreeze capacity fully matured. Thus, while it is generally believed that the Gadidae evolved in the Northern Hemisphere, an assumption based primarily on their greater species diversity in northern waters (Svetovidov 1948), the zoogeographic dispersement of antifreeze types hints that these families with bipolar representation originated in the Southern Hemisphere.

Gene Amplification

Recent investigations into the genomic organization of antifreeze genes are beginning to offer additional insights into the dynamics of antifreeze evolution. Studies on the winter flounder, one of at least several species of righteye flounders which inhabit the oceans of high northern latitudes and produce similar antifreeze types (DeVries 1980), reveal a large antifreeze multigene family of approximately 40 members (Davies et al. 1984). Remarkably, the majority of these genes are arrayed in direct tandem repeats (Scott et al. 1985). This pattern of gene organization is extremely rare, having been described for some sets of histone genes (Shaffner et al. 1976), ribosomal RNA genes (Long and Dawid 1980), and for the tubulin genes of trypanosomes (Thomashow et al. 1983).

Gene amplification in response to intense evolutionary pressure has been demonstrated in the laboratory in the classic example of cells exposed to sublethal doses of methotrexate (Alt et al. 1978). It seems natural to explain the flounder's tandemly iterated antifreeze genes as arising from an amplification event driven by Northern Hemisphere Cenozoic cooling (Scott et al. 1985). Given the definitive signature of the flounder's iterated antifreeze gene arrangement (Fig. 3a), comparison with the organization of the homologous gene in a fish closely related to the winter flounder should provide a very sensitive measure as to when, and perhaps by what mechanism, antifreeze gene amplification occurred. Preliminary results on *Limanda ferruginea* and *Glyptocephalus cynoglossus*, other righteye flounders from the same region but living in deeper water, indicate the presence of several homologous antifreeze genes with a dispersed rather than tandemly iterated pattern (Fig. 3a). This suggests that tandem amplification of the winter flounder's antifreeze genes is a recent event, postdating the divergence of these closely related flounders.

Continued investigation into the genomic organization of antifreeze protein genes may well reveal amplification as the hallmark of antifreeze evolution. Figure 3b shows a genomic Southern blot of ocean pout DNA probed with antifreeze protein cDNA compared with an identical blot probed with a cDNA to a presumed single copy gene. Clearly the antifreeze protein genes in this species constitute an exceptionally large multigene family. By analogy with the winter flounder situation, analysis of homologous genes in near relatives of the ocean pout should provide a framework to decipher its antifreeze evolution.

Conclusion

Whereas the lineage of the more commonly studied proteins such as globins, crystallins, and cytochrome *c* can be projected back 10^8 to 10^9 yr ago, at least some of the fish antifreezes developed as recently as 10^6 to 10^7 yr ago. The study of their genes may provide us with a unique opportunity to investigate the evolution of multigene families which are still in a state of flux.

Acknowledgements

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A Distance Constraint Model for the Prediction of Protein Tertiary Structure

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Given only the primary structure of a globular protein, the tertiary structure is predicted using current nonlinear programming techniques. The model is constructed in the geometrical space of distances between points. The residues are positioned by the minimization of a simple function of their hydrophobicity classes, given constraints on their near-neighbour distances and bounds on their far-neighbour distances. Disulfide bond information or chemically derived substructures may also be utilized, where appropriate. Tertiary structures for pancreatic trypsin inhibitor and lysozyme are calculated, and are found to have global structures similar to those from X-ray diffraction studies. The model is compared with other distance constraint models, and properties of this class of model are explored.

À partir seulement de la structure primaire d'une protéine globulaire, on prédit la structure tertiaire en utilisant les techniques courantes de programmation non linéaire. Le modèle est construit dans l'espace géométrique formé par les distances entre les points. Les résidus sont positionnés par minimisation d'une fonction simple de leur classe d'hydrophobicité, compte tenu des contraintes sur les distances de leurs voisins immédiats et des limites sur les distances de leurs voisons plus éloignés. On peut également utiliser, au besoin, l'information sur les liaisons disulfure ou les sous-structures dérivés chimiquement. On calcule les structures tertiaires de l'inhibiteur de la trypine pancréatique et de la lysozyme et on trouve qu'ils ont des structures globales semblables à celles trouvées au cours d'études de la diffraction par les rayons X. On compare le modèle aux autres modèles de contraintes spatiales et on examine en général les propriétés de cette classe de modèle.

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Prediction of the three-dimensional structure of proteins is currently a central topic of interest in molecular biophysics. This problem is simple to define, and yet has eluded a complete solution despite nearly 30 yr of continuous study.

A single-strand protein may be envisioned as an open polygonal arc in R^3. It consists of a sequence of standardized units, amino acids, which are joined by a standardized linkage, the peptide bond. There are only 20 naturally occurring amino acids. By the nature of the peptide bond, all proteins are nonbranching chain molecules. The atomic groups of the amino acid residues that form the backbone of the chain are uniform, so for a protein of a specified length all information is restricted to the side chains of the residues. An individual protein can thus be uniquely identified by specifying the number and sequence of its amino acid residues, called its primary structure.

A protein, defined by its primary structure, will fold spontaneously into a unique stable three-dimensional structure in a suitable environment. The tertiary structure of the protein is defined as the positions of its atoms on R^3. The problem then, simply put, is to determine a mapping that produces the unique tertiary structure, given the primary structure. Secondary structures are regular geometrical hydrogen-bonded patterns, such as helices, β-pleated sheets or strands, and hairpin turns. These identifiable substructures, intermediate between primary and tertiary structures, will not be of direct concern in this model.

The spontaneous generation of tertiary structure is accomplished as the molecule, in conjunction with its surrounding medium, follows an energy gradient to a minimum free-energy state.

It would appear that the straightforward approach of minimizing the equations describing the free-energy state of a protein would be the best way to solve the tertiary structure problem. However, this method has been found to be plagued with difficulties (Nemethy and Scheraga 1977). The equations are nonlinear and the free-energy surface is pocketed with many deep local minima. Some parameters cannot be accurately estimated. A further complication is that it is unclear whether a protein's free-energy state in vivo corresponds to a global minimum or a strong local minimum. Attempts to grossly simplify the free-energy function or to minimize energy-like functions (Levitt and Warshel 1975; Levitt 1976) have also met with limited success.

It appears unlikely that free-energy equations can be solved in any real sense in the near future. Therefore, one must search for alternate means of tackling this problem.

The crystalline tertiary structures of many globular proteins are now available from the laboratory technique of X-ray diffraction. This technique is extremely costly, but is thought to produce a good average of a protein's in vivo structures. An alternative approach from direct free-energy minimization is to use empirical and statistical methods to exploit the infor-

mation from X-ray diffraction studies. Universal geometric characteristics are sought by examining known tertiary structures. The tertiary structure of an unresolved protein may then be predicted by forcing its residues into a configuration sharing the properties of the known structures.

It has been shown that the geometry of all globular proteins are found to contain common structural restrictions, which are naturally expressed as constraints on distances between pairs of points. Tertiary structure prediction models using these methods have shown much promise in recent years (Ycas et al. 1978; Goel and Ycas 1979; Goel et al. 1982; Cariani and Goel 1985; Kuntz et al. 1976, 1979; Crippen 1977; Havel et al. 1979; Havel and Wüthrich 1984; Wako and Scheraga 1981, 1982). They are variously referred to as distance constraint, distance geometry, or semi-empirical models.

The distance constraint model to be specifically discussed in this paper used the model of Goel and Ycas (1979) as its starting point. However, it differs from previous models in several respects apart from the number and type of geometrical constraints involved. It is formulated as a nonlinear optimization problem. It was deliberately constructed to be suitably constrained with respect to both Cartesian coordinates and the coordinates defined by pairwise distances between points. Further, the solution algorithm was specifically designed for the problem. As with other distance constraint models, it does not attempt a high-resolution prediction of the tertiary structure, only correct global characteristics.

With distance constraint models, a small number of simple controls force the protein into its final tertiary structure. These models do not attempt to follow the folding process in any way, but when the constraints are well chosen, they do reflect the underlying dynamics of the folded state of the protein. We could say the free-energy equations are implicit in the empirical constraints. Also, by using known native protein structures as their basis, the question of whether the real folded protein lies at a local or global free-energy minimum is irrelevant for distance constraint models.

The Constraints

A protein will be represented by the positions of the central C^{α} atoms of its residues, connected by virtual bonds. Although information is obviously lost whenever a protein is represented in this manner, the nonlinear programming model will compensate for this simplification to some extent. For instance, hydrophobicity conditions and lower bound constraints for pairwise distances between residues in the model will implicitly allow for the positions of the side chains.

The residues (i.e. the C^{α} atoms) will be positioned by imposing a very simple set of constraints on the geometry of the molecule. All of these constraints, which arise naturally from empirical observation of known tertiary structures, are in the form of distances between pairs of points. The points may be the positions of the residues or, like the centroidal point, a functional combination of positions of the residues.

Part of the task when formulating a distance constraint model is to choose the set of constraints to be used. There are several criteria for the choice of a constraint set: (1) availability from the primary structure, (2) accuracy of the constraint, (3) degree of control on the system that the constraint would exert were it known exactly, and (4) redundancy. The degree of accuracy of the constraint refers to a measure of the variability associated with the constraint parameter when predicting from primary structure.

Possible candidates for inclusion in a distance constraint model are given in Table 1. The various types of distance constraints fall into two classes: (1) local residue−residue pairwise distances and (2) global (i.e. long range with respect to primary sequence) residue−residue or centroidal point−residue distances. Some distance constraints of the global type are chemically derived and cannot be found directly from the primary structure.

The local peptide geometry determines the near-neighbour distances for the residues. The first-neighbour distances are found to be essentially constant from both theoretical and empirical perspectives, and it is this constraint that gives the protein its chain structure. Pairs of residues that are close together in the primary structure, but not adjacent, can have a range of distance separations which vary within definite bounds. The minimum bounds on these distances are determined by steric hindrance and van der Waals forces. These minimum bounds are best estimated by empirical methods, and the model in this paper will use values found previously (Goel and Ycas 1979) from X-ray diffraction data of 21 globular proteins. The mean values for these distances are also best determined empirically, since X-ray diffraction errors will be averaged by this calculation and any theoretical procedure would involve an empirical evaluation of secondary structure proportions. The maximum bounds for the distances are best determined theoretically by considering chemical bond lengths and angles, to avoid using measurement errors from the X-ray diffraction data. Usually, only second- to fourth-neighbour distances are used in distance models, as distances between residues farther apart in the chain show greater variability and provide little additional information.

There is little correlation for mean values of far-neighbour distances for proteins, although far-neighbour residues show a definite minimum distance of approach due to van der Waals forces. They also show a definite maximum distance of separation, which is a function of the length of the primary sequence and is due to the packing of the globular molecule.

A very effective empirical constraint for determining protein structure is the so-called "hydrophobicity" rule (Danielli 1938; Kauzmann 1959). This rule separates the 20 amino acids into their tendencies to lie in the interior or on the exterior of the molecule, due to the chemical properties of their side chains. The rule is only approximate, but is valuable in that it provides global information about the tertiary structure. It can easily be expressed in terms of distance measurements between each residue and the centroidal point of the molecule: J_1: hydrophobic, tends toward the centroidal point; J_2: hydrophilic, tends toward the outer surface; J_3: ambivalent, has no tendency. In the present model, the classification of Goel and Ycas (1979) is used. They divided the 20 naturally occurring amino acids into three hydrophobicity categories by semi-empirical methods as follows: hydrophobics: val, leu, ile, phe, met; hydrophilics: arg, asa, glu, gln, gly, lys, pro; ambivalent: ala, asp, cys, his, ser, thr, try, tyr. Although there are numerous hydrophobicity indices for the amino acids based on energy or chemical considerations (Wertz and Scheraga 1978; Charton and Charton 1982), it is felt that classifying the amino acids on a simple empirical basis is best. Keep in mind that the goal is to predict the final folded structure, not to mimic the folding process.

Disulfide bonds are cross-links between pairs of cysteine residues, which may be far apart in the primary sequence. These bonds are probably not integral to the folding process, but certainly aid in the stability of the folded protein. Knowledge of the primary structure locations of the residues participating in a disulfide bond can greatly improve the performance of distance model predictions. Unfortunately, disulfide bond locations are usually inaccessible from primary structure alone. However, estimates of pairwise distances from disulfide bonding, bifunctional reagent bonding (Wold 1981), nuclear Overhauser measurements (Braun et al. 1981), or other physicochemical techniques (Cohen and Sternberg 1980a) can provide a valuable source of far-neighbour information. This information may be vital for the distance model to be successful (Havel et al. 1979; Wako and Scheraga 1981; Goel et al. 1982).

The Mathematical Model

The mathematical model employed in the present study is formulated with several objectives in mind. A model should incorporate the information from the semi-empirical distance constraints so as to remain simple in form and easy to modify. Equally important, it must be capable of methodically obtaining solutions (i.e. predicted tertiary structures) for an entire class of primary structures. It must be suitably constrained, in the sense to be described in the next section, so that the predicted tertiary structures are not dependent upon the imposed initial prefolded conditions.

The model is written as a nonlinear programming problem, which is described in the Appendix. The hydrophobicity condition is the objective function of the problem. The programming constraints consist of first-neighbour mean distances (and usually second-neighbour), minimum and maximum bounds for other near-neighbour and far-neighbour distances, and disulfide bond distances where applicable. The values of the parameters used in the model are also discussed. The model is formulated in terms of distance geometry coordinates, since this method most accurately reflects the empirical constraints.

Suitably Constrained Systems

Any mathematical model formulated in distance geometry coordinates (called Y-space) must satisfy three conditions in order to yield useful results: (1) Accurate solutions y^* of the mathematical model (i.e. tertiary structure predictions) must be achievable in practice. (2) The dimensionality of all possible solutions y^* must be small. (3) The domain of the solution space must be small with respect to the usual Cartesian coordinates in R^3. Denoting this space by X-space, we can say that the dimensionality of the solutions x^* in X must be small.

Condition 1 simply states that there must exist an algorithm that is guaranteed to systematically find a solution, given a set of initial conditions. This condition may seem trivial, but is decidedly nontrivial with large-scale nonlinear systems such as these.

If condition 2 does not hold, then the system is underconstrained and the predicted tertiary structures will be found to be dependent upon the initial conditions. Different initial configurations will produce y^* values in divergent areas of the solution space.

Condition 3 refers to local continuity properties of the mapping ψ: $Y \rightarrow X$ (Rosen 1980). Let us measure differences in structures with respect to Y-space by the distance root-mean-square metric:

$$\mathrm{RMS}_y = \left[\frac{1}{N}\sum_{(i,j)} (d - d')^2\right]^{1/2}$$

where d and d' are the set of respective $d_{i,i+j}$ distances for the two structures, N is the total number of residue pairs, and the summation is over all pairs of residues. Differences in structures with respect to X-space coordinates will be measured by the X-space root-mean-square metric:

$$\mathrm{RMS}_x = \left[\frac{1}{3n}\sum (x - x')^2\right]^{1/2}$$

where x and x' are the usual Cartesian coordinates for the two structures, $3n$ is the number of coordinates in a protein of length n, and the summation is over all coordinates. It is easily seen that small perturbations of a structure in Y-space as measure by RMS_y can produce large-scale changes in the structure with respect to the X-space coordinates as measured by RMS_x. One purpose of a distance geometry model must be to eliminate all such perturbations, since, clearly, the existence of dissimilar optimized structure predictions x^* for a single protein is unacceptable.

To quantify conditions 2 and 3, we must investigate the mathematical form of the transformation ψ: $Y \rightarrow X$ and the effect of distance geometry models on ψ. In order to derive the mapping ψ: $Y \rightarrow X$, let us assume we possess the complete set of Y-space coordinates for a protein of length n. It is sufficient to know $4n - 10$ coordinates, consisting of independent elements of a set of d_{ij}, in order to determine the complete coordinate set. A mapping ψ was derived originally by Crippen (1977), and can be written as

$$(x_1, y_1, z_1) = (0, 0, 0)$$

$$(x_2, y_2, z_2) = (d_{12}, 0, 0)$$

$$(x_3, y_3, z_3) = \left(\frac{d_{13}^2 - d_{23}^2 + d_{12}^2}{2d_{12}}, [d_{13}^2 - x_3^2]^{1/2}, 0\right)$$

$$(x_4, y_4, z_4) = \left(\frac{d_{14}^2 - d_{24}^2 + d_{12}^2}{2d_{12}}, \frac{d_{14}^2 - d_{34}^2 + d_{13}^2 - 2x_3x_4}{2y_3}, [d_{14}^2 - x_4^2 - y_4^2]^{1/2}\right)$$

$$(x_i, y_i, z_i) = \left(\frac{d_{1i}^2 - d_{2i}^2 + d_{12}^2}{2d_{12}}, \frac{d_{1i}^2 - d_{3i}^2 + d_{13}^2 - 2x_3x_i}{2y_3}, \pm [d_{1i}^2 - x_i^2 - y_i^2]^{1/2}\right) \quad i = 5, \ldots, n.$$

Crippen also noted that the mapping is not stable. For example, if $d_{13} \cong d_{23} \gg d_{12}$, then a small perturbation in any of these Y-space coordinates would cause a large change in the value of x_3. A distance geometry model will act to reduce the effective degrees of freedom in Y-space, and a good model must eliminate the instabilities in the mapping.

It must be noted here that Sippl and Scheraga (1985) have since completely solved the mapping ψ: $Y \rightarrow X$, effectively resolving the troublesome $\pm$ term for z_i in Crippen's mapping. However, this section is more concerned with the local con-

TABLE 1. Possible constraints for model.

Local
First-neighbour mean distance
Near-neighbour mean distances
Near-neighbour minimum distances
Near-neighbour maximum distances
Secondary structure predictions
Global
Far-neighbour mean distances
Far-neighbour minimum distance
Far-neighbour maximum distance
Hydrophobicity index of residues
Chemically derived:
disulfide bond mean distance
chemical modifications
(bifunctional reagents, etc.)

tinuity properties of ψ, to show that conditions 2 and 3 hold under the present model. For this exercise, Crippen's mapping will serve as a good example.

Let us assume that an optimum point $y^* \in Y$ has been reached with respect to the model. A small perturbation $y^* \rightarrow y'$ should serve to push the configuration to a nonoptimum one (condition 2). If the model is suitably constrained, the change in structure with respect to X-space should also be small (condition 3).

Unfortunately, under most models a perturbation $y^* \rightarrow y'$ will affect the X-space coordinates of the first four residues, as well as those of (x_i, y_i, z_i). However, since Crippen's equations will hold for any four points chosen as four coordinate references, we may choose the following: $(0, 0, 0)$: centroidal point of the molecule; $(x_2, 0, 0)$: p_{i-1}, the nearest neighbour to residue p_i; $(x_3, y_3, 0)$: p_{i-2}, the second neighbour to residue p_i; (x_4, y_4, z_4): p_{i-3}, the third neighbour to residue p_i. Then, if the model requires exact conditions on the centroidal point, first-neighbour, and second-neighbour distances at equilibrium, the X-space coordinates of these four reference points will be invariant under any perturbation $y^* \rightarrow y'$. Under an arbitrary perturbation, the change in the point p_i in X-space coordinates will be given by

$$x_i' - x_i^* = \frac{(d_{1i}'^2 - d_{1i}^{*2}) - (d_{2i}'^2 - d_{2i}^{*2})}{2d_{12}}$$

$$y_i' - y_i^* = \frac{(d_{1i}'^2 - d_{1i}^{*2}) - (d_{3i}'^2 - d_{3i}^{*2})}{2y_3} - \frac{x_3}{y_3}(x_i' - x_i^*)$$

$$z_i' - z_i^* = \frac{(d_{1i}'^2 - d_{1i}^{*2}) - (d_{4i}'^2 - d_{4i}^{*2})}{2z_4} - \frac{y_4}{z_4}(y_i' - y_i^*) - \frac{x_4}{z_4}(x_i' - x_i^*).$$

Under the requirements of the model, this gives

$$x_i' - x_i^* = 0$$

$$y_i' - y_i^* = 0$$

$$z_i' - z_i^* = \frac{d_{4i}^{*2} - d_{4i}'^2}{2z_4}.$$

The last expression simply reduces to $z_i' = \pm z_i^*$. Thus, any perturbation $y^* \rightarrow y'$ under this model will limit the perturbations possible in X-space to a set of measure zero. The addition of further min and max constraints to the model may limit the allowable perturbations in Y-space to zero. Thus, the model as written in the Appendix is suitably constrained.

When the model does not include exact second-neighbour constraints, analytical results are extremely difficult to obtain. The argument must proceed heuristically that conditions 1 and 2 are satisfied. A test for such a model is to optimize the same protein from several random starting configurations, and to further test the model by optimizing with slightly changing parameter values from the same initial configuration.

The Algorithm

The distance constraint model is formulated as a nonlinear programming problem, which in turn is solved using a penalty function $p(x, \mu)$. The programming problem and the penalty function are given in the Appendix. The overall problem is solved by alternately minimizing $p(x, \mu)$ for a fixed value of μ and reducing μ. The expression $p(x, \mu)$ is nonconvex, and therefore an overall solution may correspond to a strong local minimum, and not to a global minimum.

The algorithm for minimizing $p(x, \mu)$ is based upon two fundamental methods of nonlinear optimization: Newton's method and steepest descent. The algorithm further employs a refinement strategy for large-scale systems, called the truncated-Newton method (Dembo and Steihaug 1983). This saves on computer execution time and memory space by calculating only an approximate solution to the Newton equations at each step, using an iterative scheme. The algorithm was designed in conjunction with the mathematical model, and fully exploits the sparsity of the Hessian matrix of second derivatives.

There is no standard procedure for solving large-scale nonlinear systems of equations, and there are not as yet standardized test problems for evaluating solution techniques. In our case, the best method was to design an algorithm which could utilize explicit second-order information of a continuously differentiable function while maintaining low storage space from the sparse Hessian.

Results

The proteins used for this study are bovine pancreatic trypsin inhibitor (BPTI) and lysozyme. Being a true globular protein with very small size, BPTI has become a standard test molecule for tertiary structure prediction models. It acts to inhibit the digestive enzyme trypsin by binding with it as a pseudo-substrate. Lysozyme was the first true enzyme to have its tertiary structure determined by X-ray diffraction (Blake et al. 1965). Its function is to dissolve certain bacteria by hydrolyzing the polysaccharide component of their cell walls.

The algorithm was implemented in FORTRAN, using the penalty function $p(x, \mu)$ and parameters as described in the Appendix. The initial configurations used were randomly generated sets of points in R^3, scaled approximately to the volume of the protein. The structures were compared with known X-ray diffraction coordinates at each stage of the optimization process (i.e. for each value μ of $p(x, \mu)$). The X-ray structures are considered the "real" structures.

The metric for comparing structures was the RMS_y. This value is the most common metric for measuring similarity of

TABLE 2. Statistical comparison of the optimized structure for BPTI (three disulfide bond constraints included) with the structure from X-ray diffraction. The X-ray diffraction structure is considered "real." The amino acids are divided into three classes: J_1 (hydrophobic), J_2 (hydrophilic), and J_3 (ambivalent). Let $d_{i,cp}$ represent the distance of residue i from the centroidal point of the molecule.

	Real		Optimized		
Distance	Mean	SD	Mean	SD	Mean difference
$d_{i,i+1}$	3.80	0.03	3.80	0.003	
$d_{i,i+2}$	6.12	0.69	5.96	0.02	
$d_{i,i+3}$	8.12	1.71	7.85	1.25	
$d_{i,i+4}$	9.94	2.48	9.53	1.72	
$d_{i,cp}$ $i \in J_1$	8.48	2.88	5.81	2.44	3.63
$d_{i,cp}$ $i \in J_2$	11.23	3.02	10.75	2.50	2.00
$d_{i,cp}$ $i \in J_3$	9.30	3.85	9.19	2.45	2.21
$d_{i,cp}$ all	9.99	3.48	9.28	2.99	2.36

TABLE 3. Statistical comparison of the optimized structure for lysozyme (four disulfide bond constraints included) with the structure from X-ray diffraction. The X-ray diffraction structure is considered "real." The amino acids are divided into three classes: J_1 (hydrophobic), J_2 (hydrophilic), and J_3 (ambivalent). Let $d_{i,cp}$ represent the distance of residue i from the centroidal point of the molecule.

	Real		Optimized		
Distance	Mean	SD	Mean	SD	Mean difference
$d_{i,i+1}$	4.32	1.47	3.80	0.01	
$d_{i,i+2}$	6.28	1.42	6.05	0.19	
$d_{i,i+3}$	7.35	2.00	8.08	1.18	
$d_{i,i+4}$	8.51	2.48	9.76	1.81	
$d_{i,cp}$ $i \in J_1$	10.10	3.34	9.09	2.78	2.95
$d_{i,cp}$ $i \in J_2$	15.12	4.11	14.99	3.12	2.61
$d_{i,cp}$ $i \in J_3$	12.63	3.44	11.66	3.82	2.70
$d_{i,cp}$ all	13.09	4.01	12.39	3.96	2.74

protein structures, mainly due to the singularity in calculating RMS_x values. Of course, this model is suitably constrained so that small RMS_y differences will also be small with respect to RMS_x. As a general guideline, if two structures are very similar they should have an RMS_y difference of 1–3, whereas an RMS_y value greater than 6 indicates that the two proteins may have dissimilar shapes globally (Cohen and Sternberg 1980b; Remington and Matthews 1980).

The results are displayed in the form of distance matrices, also called contact maps (Levitt 1976; Ycas et al. 1978). In these displays, the elements of the Y-space coordinate matrix for a protein are shown symbolically. In Fig. 1 and 2, if $d_{ij} < 3.0$ for some i and j, the position for d_{ij} in the matrix will be blank; if $d_{ij} > 10.0$, the corresponding position will be denoted by a period; if $3.0 < d_{ij} < 10.0$, the position will be denoted by a coded letter symbol. Each protein structure shows a characteristic pattern on a contact map. Secondary structures are readily identifiable. An α-helical configuration shows as a broad band along the diagonal because it results in small near-neighbour distances. Antiparallel β-strands show as a band running perpendicular to the main diagonal. Since the matrix is symmetric, only one half of the matrix needs to be shown. For comparison purposes the contact maps of Fig. 1 and 2 will always show the real structure below the main diagonal, and the structure to be compared above the diagonal.

In Fig. 1 are given the contact maps for the optimization of BPTI, including the three disulfide bond constraints. The optimized structure compares quite well with the real structure. Both contact maps show the α-helix for residues 45–58, and antiparallel structures for residues approximately 2–14 versus 14–26. The optimized structure does not predict the magnitude of the antiparallel structure for residues 4–26 versus 26–43. The RMS_y values were found to be 11.61 for the initial configuration, 4.96 after the first penalty function loop, and 4.22 for the optimized structure (i.e. after four loops). Further results concerning this structure are given in Table 2.

Figure 2 shows contact maps for the optimization of BPTI where the disulfide bond constraints have been removed. In the optimized structure, many of the secondary structures are now absent. The α-helix at 45–58 and the antiparallel structure at 4–26 versus 26–43 are both missing, although the short antiparallel structure at 2–14 versus 14–26 is present. The RMS_y values in this case are 13.40 for the initial configuration, 5.78 after the first loop, and 5.58 after the final loop.

It is seen that the optimized structure is considerably improved by the inclusion of the disulfide bond locations. This shows the importance of far-neighbour constraints for these distance constraint models. It appears that some chemically derived far-neighbour distance information may be crucial for these models to consistently generate accurate predictions.

The lysozyme molecule contains 129 residues, and is therefore much larger and more structurally complex than BPTI, with 58 residues. However, with the penalty function in its present form, the optimizing algorithm is capable of handling second-order information to find strong optima for proteins several times as long as lysozyme. The structure of lysozyme was optimized with the inclusion of the four disulfide bond constraints. The contact map of the optimized structure (not shown) shows a pattern generally similar to the real structure, with some notable differences in proximal residues near the active site. The RMS_y comparisons were found to be 16.15 for the initial configuration, 6.37 after the first loop, and 5.75 for the final structure. Further comparisons between the optimized structure and the real structure are found in Table 3. From the $d_{i,i+1}$ comparisons it is seen that the X-ray diffraction data employed is slightly in error, with a mean value of 4.32 Å (1 Å = 0.1 nm) instead of the expected 3.80. Also, the convergence of the optimized structure is shown to be incomplete from the standard deviation results of 0.01 Å for $d_{i,i+1}$ and 0.19 for $d_{i,i+2}$. The centroidal point distances for the residues of the optimized structure are very close pointwise to their counterparts in the X-ray diffraction structure, differing by an average of only 2.74.

The results compare well with those of other models, although direct comparison is difficult. Other models that rely on distance constraints are often concerned with the properties of distance constraint information and the testing of their algorithms. Usually, varying amounts of information about the protein to be folded are included a priori. For the purposes of this model, we wish to limit the reported results to those attained using parameters that are nonspecific to the protein in question, and not discuss the various other objectives and results of other models.

The approach of Goel and co-workers (Ycas et al. 1978; Goel and Ycas 1979; Goel et al. 1982; Cariani and Goel 1985) attempts to satisfy identically a set of distance constraints by

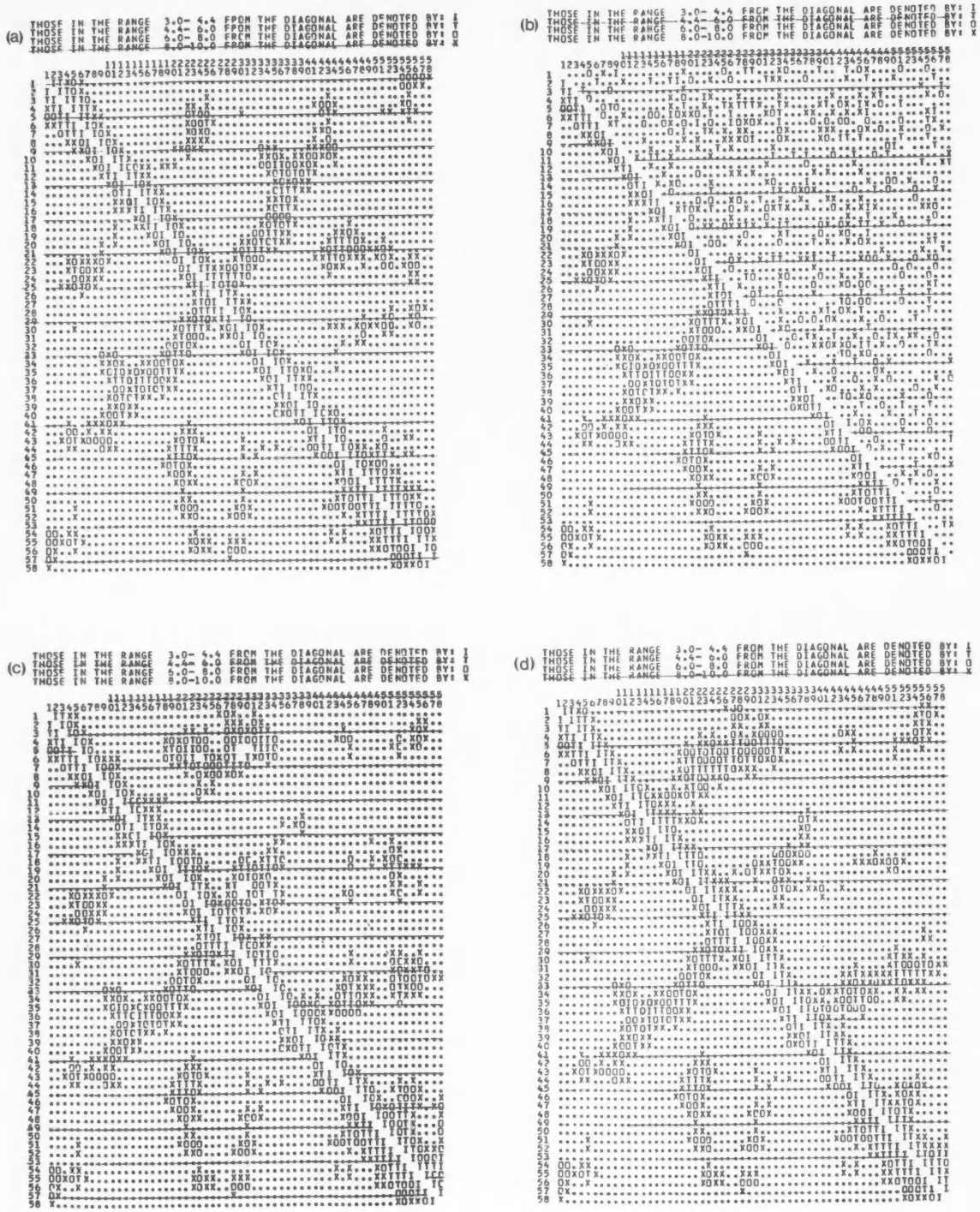

FIG. 1. Contact maps for the optimization of BPTI, including disulfide bond constraints. (a) Real structure; (b) initial configuration; (c) structure after first loop; (d) optimized structure.

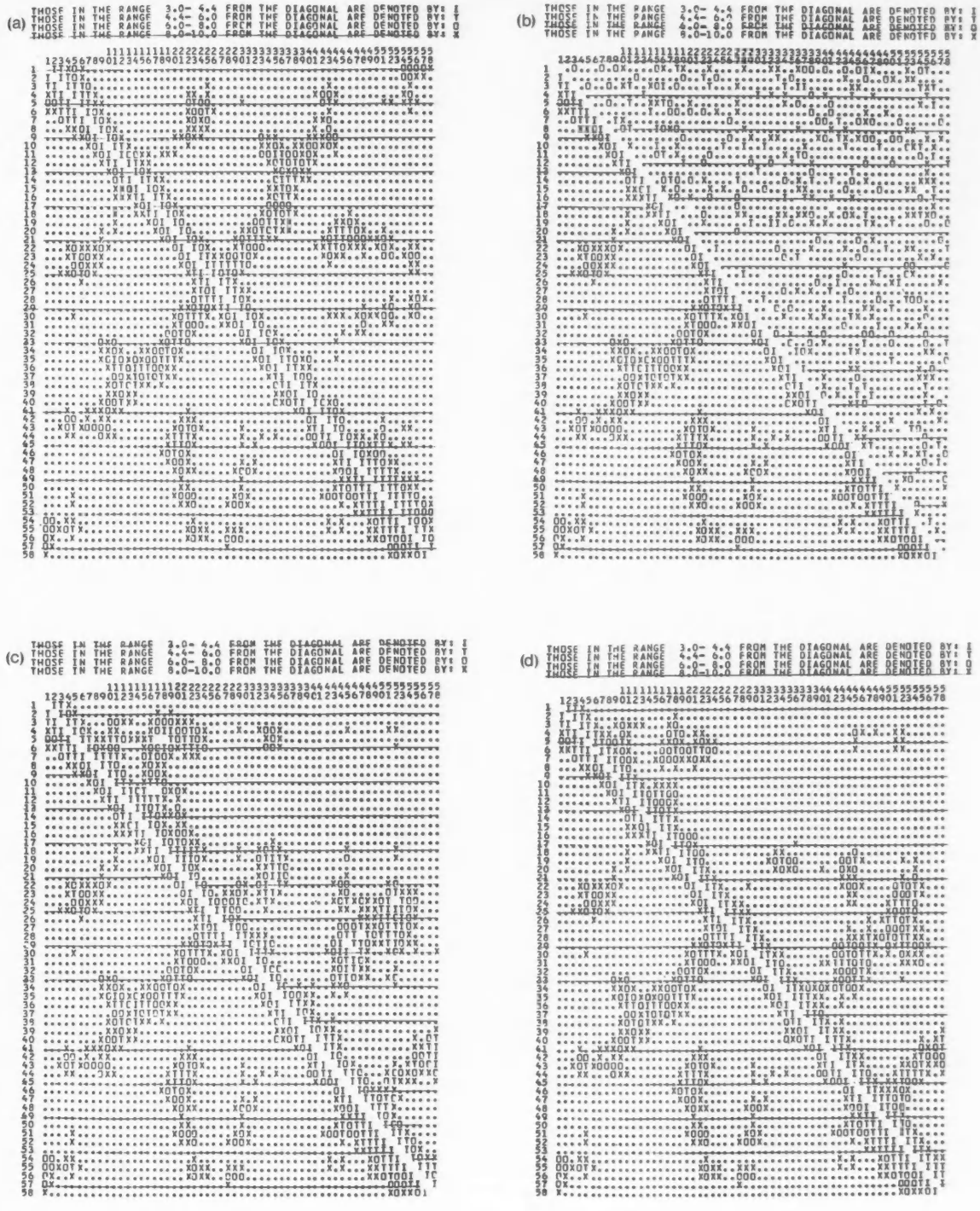

FIG. 2. Contact maps for the optimization of BPTI, excluding disulfide bond constraints. (a) Real structure; (b) initial configuration; (c) structure after first loop; (d) optimized structure.

writing the constraints in the form of a weighted penalty function. Various constraint combinations are presented to be exactly solved with the constraints being either pairwise distance constraints, min or max bounds on distances, or set average constraints. The set averages are weak constraints requiring a set of residues to attain an average distance, with no restrictions on individual distances. The penalty function is solved by minimization in the corresponding Euclidean space coordinates. A sequential optimization is performed where each residue is selected in turn and its position optimized while keeping the other residues fixed. It is known that this type of optimization can lead to a poor convergence rate and local minima trapping or cycling. Goel and Ycas (1979) found RMS_y values of 4.8–5.8 for BPTI, using large perturbations of the real BPTI structure for their initial configurations. Goel et al. (1982), using an underconstrained system folded from a random chain input, obtained an RMS_y value of 6.80 for parvalbumin, a globular protein with 108 residues and no disulfide bonds. They further obtained RMS_y values of 4.86 for BPTI and 5.21 for parvalbumin by using an overconstrained system from a similar input. In all cases, their results were found to be dependent upon the choice of initial configuration, indicating unsuitably constrained systems.

The approach of Kuntz and co-workers (Kuntz et al. 1976, 1979; Crippen 1977; Havel et al. 1979; Havel and Wüthrich 1984) is to first impose a set of pairwise distance constraints directly in *Y*-space. The system is easily solved with respect to this coordinate system by simply assigning values in the distance matrix. The difficulty arises in making the transformation of the optimized structure back into *X*-space. Unfortunately, due to properties of the distance coordinates, an arbitrary distance matrix will correspond to a structure in R^n, where $n > 3$. Hence, the system is essentially overconstrained. The most difficult step of this approach is to decide in some rigorous fashion which distance constraints to relax so that the distance matrix can be embedded in R^3. Kuntz et al. (1979) report RMS_y values of 5.46 ± 0.28 for BPTI, although they add that their best results are in the range of 3.75–4.25. In this study, not only were the structures repeatedly generated, but an RMS_x value was calculated as well.

Semi-empirical estimates for the disulfide distances in BPTI were included as constraints in all the above models.

Discussion

Distance constraint models are found to be valuable as reliable low-resolution predictors of protein tertiary structure. All that is required for input in these models is the primary structure of a protein, although the results can be generally improved by the inclusion of constraints representing disulfide bond locations or other information concerning distances between residues that are far apart in the primary structure.

These models certainly are not as yet optimal with respect to the constraint set imposed and to the mathematical form of the problem. All distance constraint models must conform to certain conditions relating to their formulation in distance geometry coordinates and to the inverse mapping into Cartesian coordinates, as discussed in this paper. In fact, one could envision a Hilbert space of distance constraint functions. The problem would then be to define a set of conditions in order to decide on a function that is "optimal."

Once these models become more efficient, they may be used to explore the entire class of primary structures that fold into stable tertiary structures, as opposed to the unstable and random coil sequences. They may also be used as first approximations to actual tertiary structures, which may be used by laboratory techniques such as X-ray studies, or by refinement predictor algorithms which could add secondary structure information. Scheraga (1983) has outlined a procedure for tertiary prediction which consists of repeated cycling between the methods of distance–constraint optimization and free-energy minimization.

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Appendix

The following notation will be used: $\bar{d}_j$ = mean distance between residues i and $i + j$; $\bar{d}_s$ = mean distance between disulfide bond pairs; L_j = minimum distance between jth nearest neighbours for small j (usually $j = 2, 3, 4$); U_j = maximum distance between jth nearest neighbours for small j; L_N = minimum distance for jth nearest neighbours for large j (usually $j > 4$); U_N = maximum distance for jth nearest neighbours for large j.

Let $S = \{(l, k) \mid$ residues l and k are connected by a disulfide bond$\}$. Let $d_{i,i+j}$ represent the Euclidean distance between residues i and j:

$$d_{i,i+j} = [(x_i - x_{i+j})^2 + (y_i - y_{i+j})^2 + (z_i - z_{i+j})^2]^{1/2}.$$

The nonlinear programming model is given as follows:

$$\text{minimize } g = \sum_{i \in J_1} d_{i,cp}^2 + \sum_{i \in J_2} (D - d_{i,cp})^2$$

subject to

$d_{i,i+j} = \bar{d}_j$; $i = 1, \ldots, n - j$ (jth nearest neighbour constraints, usually $j = 1, 2$)

$d_{l,k} = \bar{d}_s$; $(l, k) \in S$ (disulfide bond constraints)

$L_j < d_{i,i+j} < U_j$ (max and min distance constraints for near neighbours; usually $j = 2, 3, 4$)

$L_N < d_{i,i+j} < U_N$ (max and min distance constraints for far neighbours; usually $j > 4$).

The hydrophobicity conditions are thus written as the objective function of the nonlinear programming problem. The hydrophobic residues are required to tend toward the centroidal point, and the residues classed as hydrophilic are required to tend toward the surface of a sphere centered at the centroidal point. The sphere has no special physical significance, the radius (D) of the sphere being chosen simply to reflect the empirical packing density of the residues.

The objective function represents an idealization of the hydrophobicity rule, even to the extent of being chemically inaccurate. Chemically, the rule follows from the polarity of the side chains of the residues; nonpolar side chains seek the hydrophobic interior of the globule (not the centroidal point) and the polar side chains seek the aqueous environment at the molecular surface. In its present form, the objective function can mathematically reflect the empirically found tendencies in terms of pairwise distances. This affects the hydrophobicity rule, without the necessity of imposing an imaginary shell separating an "inside" from an "outside" of the molecule. The hydrophobicity rule is presented as the objective function and not in the constraint set of the model because the residues are not individually fixed at any specified distance from the molecule's center.

TABLE A.1. Values of the parameters used in the mathematical model. The values of $\bar{d}_1$, $\bar{d}_2$, $\bar{d}_s$, L_2, L_3, L_4, and L_N are taken from the semi-empirical results of Goel and Ycas (1979). The values for U_2, U_3, and U_4 were calculated theoretically by the author from chemical bonding considerations, using Ramachandran limits on the dihedral angles (ϕ, ψ). The values for U_N and D were found by the author using semi-empirical methods. Let n_1 and n_2 represent the number of hydrophobic and hydrophilic residues, respectively, in the protein of length n.

Mean value parameters	$\bar{d}_1 = 3.80$	$\bar{d}_2 = 5.95$	$\bar{d}_s = 5.46$
Lower bound parameters	$L_2 = 4.65$	$L_3 = 4.33$	$L_4 = 4.39$
	$L_N = 5.00$		
Upper bound parameters	$U_2 = 7.20$	$U_3 = 10.70$	$U_4 = 13.50$
	$U_N = 9.91n^{1/3} - 8.75$		
Centroidal point distance parameter	$D = \frac{n_1 + n_2}{n_2}(2.8n^{1/3} - 0.89)$		

The model will be solved in X-space. To this end, the centroidal point is set at the origin in this coordinate system. The model will be solved by a quadratic loss penalty function:

$$\text{minimize } p(x, \mu) = \mu g + \left(\sum_{i=1}^{n} x_i\right)^2 + \left(\sum_{i=1}^{n} y_i\right)^2 + \left(\sum_{i=1}^{n} z_i\right)^2 \text{ (centroidal point penalties)}$$

$$+ \sum_{j=1}^{2} \sum_{i=1}^{n-j} (\bar{d}_j - d_{i,i+j})^2 \text{ (jth nearest neighbour penalties)}$$

$$+ \sum_{(l,k) \in S} (\bar{d}_s - d_{l,k})^2 \text{ (disulfide bond penalties)}$$

$$+ \sum_{j=3}^{4} \sum_{i=1}^{n-j} [\min (0, U_j - d_{i,i+j})]^2 \text{ (near neighbour max distance penalties)}$$

$$+ \sum_{j>4} \sum_{i=1}^{n-j} [\min (0, U_N - d_{i,i+j})]^2 \text{ (far neighbour max distance penalties)}$$

$$+ \sum_{j=3}^{4} \sum_{i=1}^{n-j} [\min (0, d_{i,i+j} - L_j)]^2 \text{ (near neighbour min distance penalties)}$$

$$+ \sum_{j>4} \sum_{i=1}^{n-j} [\min (0, d_{i,i+j} - L_N)]^2 \text{ (far neighbour min distance penalties).}$$

The overall solution is found by alternately minimizing $p(x, \mu)$ for a fixed value of μ and reducing μ. In practice, about four loops are executed in this way, reducing μ to a tenth of its former value each time.

The values for the parameters used in this model are given in Table A.1. When fixing parameter values, the question

arises whether to attempt a theoretical derivation from first principles or to estimate the values statistically from a database of the known tertiary structures of proteins. It was argued in the constraints section of this paper that theoretical calculations are valuable in estimating upper bound distance parameters, whereas it is most reasonable to estimate mean value and lower bound distance parameters semi-empirically. This is done, using the results of Goel and Ycas (1979).

The parameter D was estimated using the empirical result of Goel and Ycas (1979) for the mean distance (angstroms) of any residue from the centroidal point for a protein of length n:

$$y = 2.80n^{1/3} - 0.89 \qquad \text{(correlation } r = 0.98\text{)}.$$

If it is assumed that hydrophobic residues (class J_1) have a tendency toward the centroidal point (i.e. to $y = 0$) and the hydrophilic residues (class J_2) tend away from the centroidal point (i.e. to $y = D$), then for the residues in classes J_1 and J_2 combined to satisfy their mean distance formula, we must have

$$D = \frac{n_1 + n_2}{n_2}(2.8n^{1/3} - 0.89)$$

where the set J_1 contains n_1 residues and J_2 contains n_2 residues of the protein.

The parameter U_N was found semi-empirically by the author. Using all possible pairs of C^α atoms in each protein, the maximum far-neighbour distances were found for the identical set of 21 proteins as used by Goel and Ycas (1979). An analysis was done on these maximum distances, where the linear least-squares regression equation and linear correlation coefficient were calculated. The results were as follows:

$$y = 9.91n^{1/3} - 8.75 \qquad \text{(correlation } r = 0.93\text{)}$$

where y is the maximum pairwise distance between C^α atoms in a protein of length n.

Evolutionary Relationship of a Fish C Type Lactate Dehydrogenase to Other Vertebrate Lactate Dehydrogenase Isozymes

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Rehse, P. H., and W. S. Davidson. 1986. Evolutionary relationship of a fish C type lactate dehydrogenase to other vertebrate lactate dehydrogenase isozymes. Can. J. Fish. Aquat. Sci. 43: 1045–1051.

It is assumed that the genes for the three types of vertebrate lactate dehydrogenase isozymes (A, B, and C) arose from an ancestral lactate dehydrogenase gene by a mechanism involving gene duplications. The currently accepted model was originally proposed by Holmes in 1972 (FEBS Lett. 28: 51–55). The main points in this proposal are as follows: (1) the ancestral lactate dehydrogenase was an A type; (2) the gene for this A type lactate dehydrogenase duplicated to produce the A and B forms; and (3) the C isozymes of fish and warm-blooded vertebrates are derived from B types by successive, independent gene duplication events. More structural data have become available since this model was first put forward, and Li et al. (1983. J. Biol. Chem. 258: 7029–7032) have shown that rodent C type lactate dehydrogenases appear to be ancestral to the A and B forms. We have extended Li's reevaluation of the evolutionary relationships among vertebrate lactate dehydrogenase isozymes. Our analysis indicates that there is no significant difference in the rates of evolution along the A, B, or C lineages. This confirms that a C type rather than an A type lactate dehydrogenase was the ancestral form. A duplication of the gene for this C type gave rise to the gene which, by a further gene duplication, yielded the A and B type lactate dehydrogenase genes. In addition, amino acid compositional data reveal that the C type lactate dehydrogenase from Atlantic cod (*Gadus morhua*) and the C type lactate dehydrogenase isozymes of rodents are homologous proteins that are the result of divergent evolution via speciation events rather than by independent gene duplications. This novel interpretation of lactate dehydrogenase isozyme evolution is discussed with respect to the tissue specificities of C type lactate dehydrogenases in vertebrates.

On présume que les gènes responsables de la formation des trois types d'isozymes (A, B et C) de déshydrogénase lactique chez les vertébrés sont issus d'un gène ancestral de déshydrogénase par un mécanisme mettant en jeu la duplication des gènes. Le modèle actuellement accepté a été proposé à l'origine par Holmes en 1972 (FEBS Lett. 28 : 51–55). Les principaux points dans cette proposition sont les suivants : (1) la déshydrogénase ancestrale était un isozyme de type A; (2) il y eu duplication du gène de ce type A de déshydrogénase pour produire les formes A et B; et (3) les isozymes de type C des poissons et des vertébrés à sang chaud proviennent de celles de type B par duplications successives et indépendantes des gènes. Davantage de données sur la structure sont devenues disponibles depuis que ce modèle a été présenté pour la première fois, et Li et al. (1983. J. Biol. Chem. 258 : 7029–7032) ont montré que les déshydrogénases de type C des rongeurs semblent être les ancêtres des formes A et B. Nous avons élargi la réévaluation de Li des relations évolutives entre les isozymes de déshydrogénase chez les vertébrés. Notre analyse montre qu'il n'y a pas de différence significative dans les taux d'évolution le long des lignées évolutives de A, de B et de C. Cela confirme qu'une déshydrogénase de type C plutôt que de type A était la forme ancestrale. Une duplication du gène responsable de ce type C a donné naissance au gène qui, grâce à une duplication ultérieure des gènes, a produit les gènes de déshydrogénase des types A et B. En outre, les données sur la composition des acides aminés révèlent que la déshydrogénase de type C provenant de la morue de l'Atlantique (*Gadus morhua*) et les isozymes de déshydrogénase de type C des rongeurs sont des protéines homologues qui sont le résultat d'une évolution divergente par le biais de la spéciation plutôt que par celui de la duplication indépendante des gènes. On discute de cette nouvelle interprétation de l'évolution des isozymes de déshydrogénase pour ce qui est de la spécificité tissulaire des déshydrogénases de type C chez les vertébrés.

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Lactate dehydrogenase (LDH) (EC 1.1.1.27) isozymes constitute a multigene family whose members are developmentally regulated (Lindsay 1963) and differentially expressed (Fine et al. 1963). They are believed to be descended from a common ancestor via a series of gene duplication events (Markert et al. 1975). The wide distribution of LDH isozymes in nature makes them an ideal system for studying all aspects of molecular evolution.

All vertebrates possess the two major types of LDH isozymes, A and B. The A isozyme is found predominantly in tissues which may undergo anaerobic glycolysis (e.g. skeletal muscle) whereas the B isozyme is the major form in tissues with an aerobic metabolism (e.g. heart) (Wilson et al. 1963). These isozymes exist as tetramers and both the homotetramers and the entire range of heterotetramers occur.

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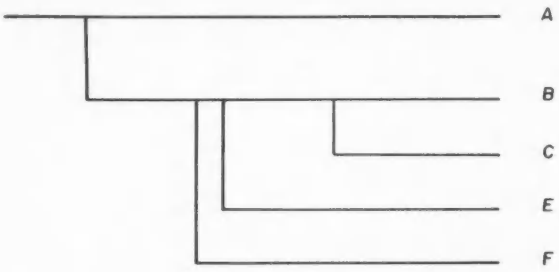

FIG. 1. Model of LDH isozyme evolution as proposed by Holmes (1972). An A type LDH is depicted as the original LDH. The B type was formed by a gene duplication of the A gene. The F (cod liver type), E (teleost eye type), and C (mammalian and bird testes type) genes are shown to have originated independently from B type genes.

Vertebrates also express a third type of LDH. In mammals and birds this isozyme is expressed only in primary spermatocytes and has been referred to as LDH-X or the C isozyme (Goldberg 1972; Zinkham et al. 1969). Many fish also have a third type of LDH which, unlike the C isozyme of warm-blooded vertebrates, is not restricted to the primary spermatocytes. In lower bony fish (e.g. sturgeon) this LDH is expressed in heart, liver, gills, kidney, and gonads (Markert et al. 1975). Most advanced teleosts limit the expression of this form of LDH to neural tissues such as the eye and brain, leading to the terms E4, eye band, and retinal isozyme 1 being used (Lush et al. 1969; Whitt 1970). In Gadiformes (e.g. cod) and Cypriniformes (e.g. carp) the liver is the tissue of choice for this isozyme. In cod, it is also found as a minor component in blood, retina, brain, and stomach (Shaklee et al. 1973). This form has been called the F4 or liver-specific LDH (Sensabough and Kaplan 1972). In keeping with the nomenclature advocated by Markert et al. (1975), we shall refer to all vertebrate LDH isozymes which are not of the A or B form as C types.

The evolutionary relationships among LDH isozymes have been the subject of research and speculation in several laboratories over the last 20 yr. The scheme which is generally accepted was first presented by Holmes in 1972, and this is illustrated in Fig. 1. It was proposed that the ancestral form of LDH closely resembled the present A type LDHs and that the gene for this protein duplicated to give rise to the A and B forms. The C type LDHs were then produced by a succession of independent duplications of the gene which coded for the B isozyme (Holmes 1972). The Holmes model has been refined (Horowitz and Whitt 1972; Shaklee et al. 1973; Holmes and Scopes, 1974; Markert et al. 1975; Whitt et al. 1975; Fisher et al. 1980) but remains essentially the same as when it was originally proposed. This model was based primarily on immunochemical studies that indicated that antibodies raised to B type LDHs cross-reacted with C type LDHs and vice versa but antibodies raised against A type LDHs did not cross-react with either the B or C types (Whitt 1969; Holmes and Markert 1969; Markert and Holmes 1969; Whitt 1970). However, the reliability of these techniques is in doubt, as Holmes and Scopes (1974) subsequently found that the A and B forms were immunochemically related. Moreover, antibodies raised to the C type LDH from mouse testes did not react with B or A type LDHs in sensitive microcomplement assays (Goldberg 1971).

The most precise method for determining the evolutionary relationships among related proteins is to compare their amino acid sequences (Wilson et al. 1977). This is now possible for three A and two B type LDH isozymes (Eventoff et al. 1977) and for the C isozymes from rat and mouse (Pan et al. 1983). Li et al. (1983) have made use of these amino acid sequences to reevaluate the evolutionary pathway leading to the A, B, and C isozymes of mammals. Their results indicate that a C type LDH was the ancestral form. This is radically different from the acknowledged view of LDH isozyme evolution (Holmes 1972; Markert et al. 1975; Holbrook et al. 1975). However, the analysis of Li et al. (1983) is incomplete in that it did not consider the possibility that there may have been an increased rate of amino acid substitution in the C type LDHs in the rodent lineage, as appears to be the case in pancreatic ribonuclease evolution (Lenstra and Beintema 1979; Beintema 1983). Moreover, the question of the relationship between mammalian and fish C type LDHs was not addressed. In this paper we present evidence that a C type isozyme was indeed the ancestral form of vertebrate LDH isozymes, and that the C type LDHs of rodents and Atlantic cod (*Gadus morhua*) are homologous proteins resulting from speciation events rather than by independent gene duplications.

Materials and Methods

Amino Acid Sequences

The amino acid sequences of LDHs that were used in this paper were taken from the literature as follows: A isozymes (pig, chicken, and dogfish) and B isozymes (pig and chicken) from Eventoff et al. (1977); C isozymes (mouse and rat) from Pan et al. (1983); *Lactobacillus casei* LDH from Hensel et al. (1983); *Bacillus stearothermophilus* and *Bacillus megaterium* LDHs from Wirz et al. (1983).

Construction of Phylogenetic Trees

The procedure of Fitch and Margoliash (1967), as modified by Prager and Wilson (1978), was used to construct and also to determine the best possible phylogenetic trees to describe the evolutionary relationships among the LDHs.

Relative Rate Test

The basis for a relative rate test has been described by Sarich and Wilson (1973). The outside group for the vertebrate LDH isozymes comprised three bacterial LDHs. The statistical methodology used to compare the different rates was a one-way analysis of variance (Steel and Torrie 1960).

Cod Liver LDH

The C type LDH homotetramer was purified to homogeneity from the liver of the Atlantic cod as described by Rehse and Davidson (1986). The amino acid composition of the cod C type lactate dehydrogenase was determined by standard methods using a Beckman 121 C single column automatic amino acid analyser.

Metzger Difference Index

The Metzger Difference Index (Metzger et al. 1968) was used to compare the amino acid compositional relatedness of several LDHs. In this method the Metzger Difference Index is calculated using the following formula:

$$\text{Metzger Difference Index} = 50\sum_{i=1}^{18} |a_i - b_i|$$

```
      1                                                                  70
PM    ATLKDQLIHNLLKEEH-VPHNKITVVGVGAVGMACAISILMKELADEIALVDVMEDKLKGEMMDLQHGSL
CM    --LKDHLIHHVHKEEHAHAHNKitvvgvgavgMACAISILMKDLADELTLVDVVEDKLKGEMMDLQHGSL
DM    ATLKDKLIGHLATSQEPRSYNKITVVGVGAVGMACAISILMKDLADEVALVDVMEDKLKGEMMDLQHGSL
PH    ATLKEKLIAPVAQQETTIPNNKITVVGVGQVGMACAISILGKSLTDELALVDVLEDKLKGEMMDLQHGSL
CH    ATLKEKLITPVAAGST-VPSNKitvvgvgqvgMACAISILGKGLCDELALVDVLEDKLKGEMMDLQHGSL
MT    STVKEELIQNLVPDKLSR--CKITVVGVGDVGMACAISILLKGLADELALVDADTDKLRGEALDLLHGSL
RT    STVKEELIQNLAPDKQSR--CKITVVGVGDVGMACAISILLKGLADELALVDADEDKLKGEALDLLHGSL
LC    ---------ASITDKDHQ--KVI-LVGDGAVGSSYAFAMLLQQIAQEIGIVDIFKDKTKGDAIDLSNALP
BS    -------MKNNGG---AR-VVVI---GAGFVGASYVFALMNQGIADEIVLIDANESKAIGDAMDFNHGKV
BM    -------MKTQFTPK-KRTVAVI---GTGFVGSSYAFSMVNQQIANGLVLIDMNKEKAEGEATDIN-GMP

      71                                                                 140
PM    FLRTPKIVSGKDYNVTANSRLVVITAGARQQEGESRLNLVQRHVNIFKFIIPNIVKYSPNCKLLVVSNPV
CM    FLKTPKITSGKDYSVTANSKLVIVTAGARQQEGESRLNLVQRMVNIFKFIIPNVVKYSPDCKllvvsnpv
DM    FLHTAKIVSGKDTSVSAGSKLVVITAGARQQEGESRLNLVQRNVNIFKFIIPNIVKHSPDCIILVVSHPV
PH    FLQTPKIVANKDYSVTANSKIVVVTAGVRQQEGESRLNLVQRNVNVFKFIIPQIVKYSPNCIIIVVSHPV
CH    FLQTHKIVANKDYAVTANSKIVVVTAGVRQQEGESRLNLVQRNVNVFKFIIPQIVkyspnciiiVVSNPV
MT    FLSTPKIVFGKDYNVSANSKLVIITAGARMVSGQTRLDLLQRNVAIMKAIVPGVIQNSPDCKIIVVTNPV
RT    FLSTPKIVFGKDYSVSANSKLVIITAGARMVSGQSRLALLQRNVTIMKAIVPVGIQNSPDCKIMIVTNPV
LC    FTSPKKIYSA-EYSDAKDADLVVITAGAPKQPGETRLDLVNKNLKILKSIVDPIVDSGFNLIFLVAANPV
BS    FAPKPVDIWHGDYDDCRDADLVVICAGANQKPGETRLDLVDKNIAIFRSIVSEVMAHGFQGLFLVATNPV
BM    FA--MK-IWAGDYKDSADADLAVITAGANQAPGETRLDLVEKNV-IFE--V---MNSGFDGIILVATNPV

      141                                                                210
PM    DILTYVAWKISGFPKNRVIGSGCNLDSARFRYLMGERLGVHPLSCHGWILGEHGDSSVPVWSGVNVAGVS
CM    diltyVAWKISGFPKHRVIGSGCNLDSARFRHLMGERLGIHPLSCHGWIVGQHGDSSVPVWsgvnvagvs
DM    DVLTYVAWKLSGLPMHRIIGSGCNLDSARFRYLMGERLGVHSCSCHGWVIGEHGDSVPSVWSGMWNA---
PH    DILTYVTWKLSGLPKHRVIGSGCNLDSARFRYLMAEKLGVHPSSCHGWILGEHGDSSVAVWSGVNVAGVS
CH    DILTYVTWKLSGLPKHRVIGSGCNLDTARFRYLMAERLGIHPTSCHGWILGEHGDSSVAVWSGVNVAGVS
MT    DILTYVVWKISGFPVGRVIGSGCNLDSARFRYLIGEKLGVNPTSCHGWVLGEHCDSSVPIWSGVNVAGVT
RT    DILTYVVWKISGLPVSSVIGSGCNLDSARFRYLIGEKLGVNPTSCHGWVLGEHGDSSVPVWSGVNVAGVT
LC    DILTYATWKLSGFPKNRVVGSGTSLDTARFRQSIAEMVNVDARSVHAYIMGEHGDTEFPVWSHANIGGVT
BS    DILTYATWKFSGLPHERVIGSGTILDTARFRFLLGEYFSVAPQNVHAYIIGEHGDTELPVWSQAYI-GYM
BM    DILAHVTQKVSGLPNGRVIGSGTILDTARFRYLLHDYFE-------LYIMGEHGDTEFPVYSHAQIGGVK

      211                                                                280
PM    LKNLHPELGTDADKEHWKAVHKEVVDSAYEVIKLKGYTSWAIGLSVADLAESIMKNLRRVHPISTMIKGL
CM    lkNLHPDMGTDADKEhwKEVHKQVVDSAYEVIKLLGYTSWAIGLSVADLAETIMKNLRRVHPISTAVKGM
DM    LKELHPELGTNKDKQDQKKLHKDVVDSAYEVIKLKGYTSWAIGLSVADLAETIMKNLCRVHPVSTMVKDF
PH    LQQLNPEMGTDNDSENWKEVHKMVVESAYEVIKLKGYTNWAIGLSVADLIESMLKNLSRIHPVSTMVQGM
CH    LQQLNPAMGTDKDSENWKEVHKQVVESAYEVIRLKGYTNWAIGLSVAELCETMLKNYLRVHSVSTLVKGT
MT    LKSLNPAIGTDSNKQHWKNVHKQVVEGGYEVLDMKGYTSWAIGLSVTDLARSILKNLKRVHPVTTLVKGF
RT    LKSLNPAIGTDSNKQEWKTVHKQVVDGGYEVLDLKGYTSWAIGLSVTDLAESILKNLKRVHAVTTLVKGL
LC    IAEWVKAHPETKEDKLVK-MFEDVRDAAYEIIKLKGATAYGIATALARISKAILNDENAVLPLSVYMDGQ
BS    PIRKLVESKGEEAQKDLERIFVNVRDAAVQIIEKKGATYYGIAMGLARVTRAILHNENAILTVSAYLDGL
BM    -LEHFINTAAIEKEPDMQHLFSQ-RDAAVTIIN-K--TYYGI-MGLVRITKAILDDENSILTVSALLEGQ

      281                                                          334
PM    YGIKENVFLSVPCILGQNGISDVVKVTLTPEEEAHLKKSADTLWGIQKELQ-F
CM    HGIKDDVFLSVPCVLGqngisdvvkvILKPDEEEQIKKSADTLWGIQKELG-F
DM    YGIKDNVFLSLPCVLNDHGISNIVKMKLKPNEEQQLQKSATTLWDIQKDLK-F
PH    YGIENEVFLSLPCVLNARGLTSVINQKLKDDEVAQLKNSADTLWGIQKDLKDL
CH    YGIQDDVFLSLPCVLSASGLTSVINQKLKDDEVAKLKKSADTLWSIQKDLKDL
MT    HGIKEEVFLSIPCVLGESGITDFVKVNMTAEEEGLLKKSADTLWNMQKNLE-L
RT    YGIKEEIFLSIPCVLGESGITDLVKVNMNTEEEALFKKSCDILWNIQKNLE-L
LC    YGIND-LIIGTPAVINRNGIQNILEIPLTDHEEESMQKSASQLKKVLTDAFAKNDIETRQ
BS    YGERD-VYIGVPAVINRNGIREVIEIELNDDEKRNFHHSAATLKSVLARAFTR
BM    YGISD-VYIGVPAIINKNGVRQIIELNLTPHEQQQLEHSASILKQTRDRAIVF
```

FIG. 2. Alignment of the amino acid sequences of vertebrate and bacterial LDHs. PM, CM, DM (A types of pig, chicken, and dogfish); PH, CH (B types of pig and chicken); MT, RT (C types of mouse and rat); LC, BS, BM (LDHs of *Lactobacillus casei*, *Bacillus stearothermophilus*, and *Bacillus megaterium*). The amino acids are given in the one-letter code. Lowercase letters indicate the most probable sequences in the parts of the chicken LDHs that have not been fully sequenced.

TABLE 1. Comparison of the amino acid sequences of lactate dehydrogenases. Data represent the number of amino acid sequence differences. PM, pig A; CM, chicken A; DM, dogfish A; PH, pig B; CH, chicken B; MT, mouse C; RT, rat C; LC, *Lactobacillus casei* LDH; BS, *Bacillus stearothermophilus* LDH; BM, *Bacillus megaterium*.

	PM	CM	DM	PH	CH	MT	RT	LC	BS	BM
PM	—									
CM	46	—								
DM	76	81	—							
PH	85	89	94	—						
CH	88	89	98	36	—					
MT	101	109	120	119	118	—				
RT	105	112	122	123	122	42	—			
LC	207	210	207	217	214	217	222	—		
BS	218	221	223	222	223	225	227	157	—	
BM	228	229	226	232	231	231	235	165	141	—

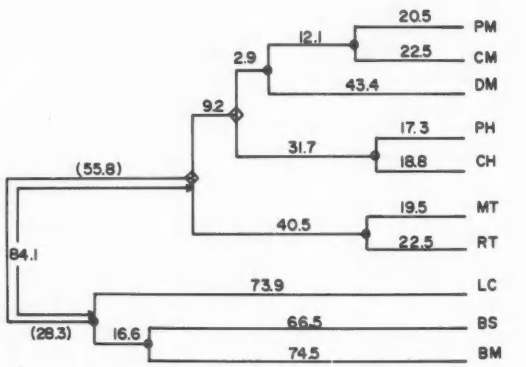

FIG. 3. Phylogenetic relationships of LDHs derived from analysis of their amino acid sequence differences. The diamonds represent gene duplications and the circles indicate speciation events. The species and LDH types are as in Fig. 2.

where a and b are the mole fractions of the ith amino acid in proteins a and b, respectively. The absolute differences between the mole fractions of the 18 amino acids in each protein are summed. Only 18 amino acids are considered, as aspartate and asparagine and glutamate and glutamine are grouped together. These pairs of amino acids cannot be differentiated in amino acid analyses. The multiplication of the sum of the absolute differences of the amino acid mole fractions by 50 gives a possible range of 0–100. It should be noted that although two sequences that are identical will have a Metzger Difference Index of 0, the converse is not true. This is because of the possibility of counterreplacements at other positions in the proteins. It is for this reason that the relationship between Metzger Difference Index and percent amino acid sequence difference must be ascertained for each set of proteins being examined.

Results

Comparison of Amino Acid Sequences of LDHs

The alignment of the amino acid sequences of pig A and B, chicken A and B, dogfish A, mouse and rat C type LDH isozymes, and three bacterial LDHs is shown in Fig. 2. Pairwise comparisons of these sequences were made and the result

TABLE 2. Comparison of amino acid sequence differences between prokaryotic LDHs and vertebrate LDH isozymes. A one-way analysis of variance indicates that there is no significant difference between these groups.

Comparison	Average	Range
Prokaryotes with A type isozymes	218.8	207–229
Prokaryotes with B type isozymes	223.3	214–232
Prokaryotes with C type isozymes	226.2	217–235

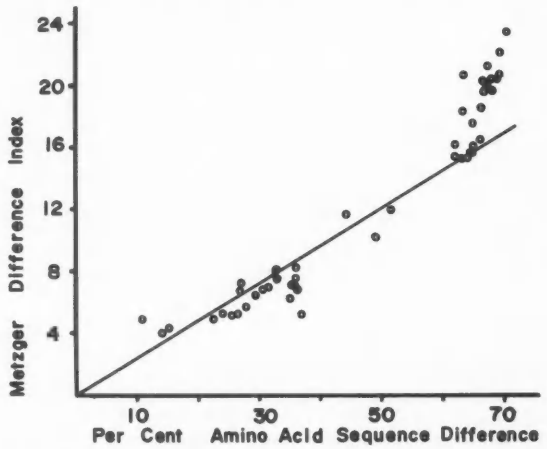

FIG. 4. Relationship between Metzger Difference Indices and their corresponding percent amino acid sequence differences for pairs of LDHs.

of this analysis is given in the form of a matrix in Table 1. The amino acid sequence data in Fig. 2 and Table 1 were used to construct a phylogenetic tree for the vertebrate LDH isozymes and the bacterial LDHs. As expected (Fig. 3), the most parsimonious tree placed the bacterial LDHs as a group quite distinct from the vertebrate LDH isozymes. The A, B, and C type isozymes each form a separate group. The rodent C type LDHs are as distantly related to the B type isozymes as they are to the A isozymes.

Relative Rate Test

One of the assumptions implicit in the type of analysis that was carried out above is that the rate of accumulation of amino acid substitutions is approximately the same along each lineage. To test whether or not this is true for the vertebrate LDH isozymes, we examined the number of amino acid sequence differences between the bacterial LDHs and each of the A, B, and C isozymes. These results are tabulated in Table 2. A one-way analysis of variance indicated that there is no significant difference in the rate of amino acid substitution along any of the vertebrate LDH isozyme lineages.

Relationship of Metzger Difference Index and Percent Sequence Difference for LDHs

There are no data concerning the structure of fish C type LDHs. Therefore, the relationship between warm-blooded C type LDHs and those of fish was investigated by comparing the amino acid compositional relatedness of several LDHs. This was achieved using the Metzger Difference Index (Metzger

TABLE 3. Amino acid composition of cod liver specific LDH.

	24[a]	48[a]	72[a]	Average	Residues/mole[b]
Asx	210.4	205.6	209.2	208.4	39
Thr[c]	118.1	97.8	104.0	121.2	22
Ser[c]	141.8	131.6	115.8	150.1	27
Glx	132.3	138.2	133.1	134.5	25
Pro	69.0	75.9	81.7	75.5	14
Gly	143.9	155.7	148.9	149.5	28
Ala[d]	100.0	100.0	100.0	100.0	19
Val	197.3	193.0	212.5	201.0	38
Ile[c]	92.4	95.4	104.9	104.9	21
Leu	181.5	184.5	180.1	182.0	35
Tyr[c]	15.1	7.8	5.8	16.5	4
Phe	27.7	28.6	26.8	27.7	6
Lys	95.7	94.0	104.0	97.9	19
His	42.6	43.1	46.6	44.1	8
Arg	72.6	77.1	75.3	75.0	14
Cys[e]	31.1			31.1	6
Met[e]	32.2			32.2	6
Trp[f]	29.3			29.3	6
Total					337

[a]Results from 24-, 48-, and 72-h hydrolysis.
[b]Assuming that 1 mol of cod C type LDH contains approximately 334 amino acid residues.
[c]Average ratios of these amino acid residues were estimated by extrapolation.
[d]Results are expressed relative to alanine which was arbitrarily set to 100 in each case.
[e]Cys and Met were determined as cysteic acid and methionine sulfone, respectively, after performic acid treatment of the protein and then a 24-h acid hydrolysis.
[f]Trp was determined after a 22-h hydrolysis using mercaptoethanesulfonic acid.

et al. 1968). To test the validity of using this method for lactate dehydrogenases, Metzger Difference Indices were calculated for the pairwise comparisons of the LDHs shown in Fig. 2. The Metzger Difference Indices were plotted against their corresponding percent amino acid sequence differences, and this is shown in Fig. 4. It appears that the Metzger Difference Index is indeed useful for analysing the evolutionary relationships among LDHs, especially those that differ by 50% or less in their amino acid sequences. Thus, information that, as yet, is not available from sequence studies may be inferred from the amino acid composition of the cod liver C type LDH.

Relationship of Cod Liver C Type LDH to Other LDH Isozymes

The liver specific C type LDH from the Atlantic cod was purified as previously described (Rehse and Davidson 1986) and its amino acid composition was determined. The number of amino acid residues per monomer of cod C type LDH is given in Table 3. This was used to determine the amino acid compositional relatedness between the cod C type LDH and LDHs whose amino acid sequences are known. The average Metzger Difference Indices between vertebrate A and B, rodent C, cod C, and bacterial LDHs (Table 4) were used to derive phylogenetic trees. The best fit for the data is given by the tree shown in Fig. 5. The C type LDHs of cod and rodents form one group. This indicates that they are orthologous proteins that are related by speciation events rather than by independent gene duplications.

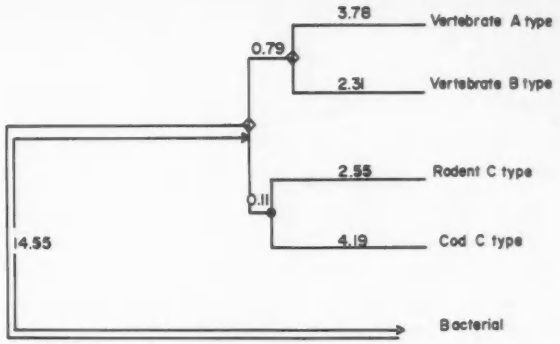

FIG. 5. Phylogenetic relationships of LDHs derived from an amino acid compositional relatedness analysis. The diamonds indicate gene duplications and the circle a speciation event.

Discussion

It has been recognised for over a decade that LDHs and their genes provide one of the best systems for studying the molecular mechanisms involved in gene evolution and gene regulation (Markert et al. 1975). Any interpretation of results at the nucleic acid level will depend upon a sound knowledge of the evolutionary relationships among the LDHs, particularly the A, B, and C isozymes found in vertebrates. Although it appeared that the phylogenetic relationships of these isozymes had been well established (Whitt 1970; Holmes 1972; Shaklee et al. 1973; Holmes and Scopes 1974; Markert et al. 1975; Whitt et al. 1975), structural studies on rodent C type LDHs (Chang et al. 1979, 1980; Pan et al. 1980) seemed to contradict the accepted dogma. When the primary structures of mouse and rat C type LDHs were determined in full (Pan et al. 1983), comparisons of these sequences with those of vertebrate A and B LDH isozymes revealed that the rodent C type LDHs were related equally distantly from the A and B types (Li et al. 1983).

One possible explanation for this observation was that there had been an increased rate of amino acid substitution and fixation in the rodent lineage. Such a situation appears to have occurred in the evolution of ribonucleases where the rate in the mouse and rat lineage is approximately 20-fold greater than in other mammals (Lenstra and Beintema 1979; Beintema 1983). However, the divergence times for rodents, as calculated from the fossil record, may have been misinterpreted and this would result in rates that are artificially high (Wilson et al. 1977; Brownwell 1983). This hypothesis can be tested by the use of a relative rate test (Sarich and Wilson 1973). The amino acid sequences of at least three bacterial LDHs have been determined (Wirz et al. 1983; Hensel et al. 1983). As the vertebrate LDH isozymes must all be equally divergent from the prokaryotic LDHs, the bacterial LDHs form an excellent outside reference group with which to test the rates of evolution along the vertebrate A, B, and C type lineages. This is verified by the tree shown in Fig. 3. The distances from the bacterial LDHs to any of the vertebrate LDH isozymes are essentially the same. The LDH isozymes are like other systems in which there has been a gene duplication event followed by divergent evolution (e.g. globins, Wilson et al. 1977; lysozyme–lactalbumin, White et al. 1977) in that the rates of point mutation fixation have been constant in the duplicated genes. Thus,

TABLE 4. Compositional relatedness of vertebrate LDH isozymes and prokaryotic LDHs (average Metzger Difference Indices).

	A types	B types	Rodent C types	Cod C type	Prokaryotes
A types	—				
B types	6.09	—			
Rodent C types	7.16	5.82	—		
Cod C type	8.93	7.34	6.74	—	
Prokaryotes	18.11	18.66	19.84	16.22	—

TABLE 5. Tissue distribution of the C type LDHs in different organisms.

Organism	Tissues
Mammals	Primary spermatocytes
Birds	Primary spermatocytes
Reptiles	Primary spermatocytes
Amphibians	Eye
Most higher bony fish	Eye
Gadiformes	Liver
Primitive bony fish	Liver, gonads, heart, gills, spleen, kidney, stomach

there has not been an increase in the evolutionary rate in the rodent C type LDHs, and the previous predictions of Li et al. (1983) are correct. This analysis refutes part of the Holmes model of LDH evolution (Holmes 1972; Holbrook et al. 1975), and brings into question the validity of the rest of it. In particular, the relationship of the fish C type LDHs to the other LDH isozymes needs to be determined.

In the absence of sequence data for fish C type LDHs, we have attempted to answer this question by making use of the Metzger Difference Index (Metzger et al. 1968) and the amino acid composition of the C type LDH isozyme from cod liver. The Metzger Difference Index has been used to compare the structural similarities among many groups of proteins including dehydrogenases (Fondy and Hollohan 1971), albumins (Wallace and Wilson 1972), lysozymes (Prager et al. 1978), aldehyde reductases (Davidson and Flynn 1980), and histones (Cote et al. 1982). Cornish-Bowden (1977) has indicated that this index can provide a significant amount of information if related proteins of the same length are compared. This is supported by the observation of Davidson and Flynn (1980) that there is a linear relationship between the percent amino acid sequence difference and the Metzger Difference Index for pairs of homologous oxidoreductases.

The evolutionary picture presented in Fig. 5 agrees with the amino acid sequence analysis of the LDH isozymes, and indicates that the cod liver specific LDH isozyme and the rodent C type LDHs are more closely related than either of these types is to the vertebrate A or B LDH isozymes. Therefore, we conclude from this result that the cod and rodent C type LDHs are orthologous proteins, i.e. they correspond to the same genetic locus and their different structures are the result of divergence due to speciation events rather than gene duplications. If this interpretation were incorrect, and the rodent C type LDH is as ancient as it appears, then one would expect to find more than three genetic loci for LDH in some vertebrates. To date, this has not been observed except in the case of some animals known to have undergone an entire genome duplication (i.e. a stable tetraploidisation, see Markert et al. 1975).

An argument that has been used to support the Holmes model of vertebrate LDH isozyme evolution is the tissue distribution of the various C type LDHs, particularly the apparent lack of a C type LDH in amphibians and reptiles. However, a non A or B type LDH isozyme has been observed using starch gel electrophoresis and zymogram staining of frog eye extracts (Lakshmipathi et al. 1980). In addition, a C type LDH has been observed in reptilian testes which are spermatogenically active (Qureshi et al. 1978). The tissue distribution of the vertebrate C type LDHs is shown in Table 5. During the course of evolution, genes encoding this LDH appear to have come under the influence of different tissue regulators. Differences in kinetic parameters (e.g. K_ms, substrate specificities, optimum substrate concentrations) among the LDH isozymes probably reflect recent adaptations to their specific cell types (Rehse and Davidson 1986). A key to understanding the function of isozymes and the control of genes in different tissues may reside in the C type LDH isozymes of fish.

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Evolution of Multifunctionalism in Enzymes: Specialist versus Generalist Strategies

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New ideas concerning nonequilibrium thermodynamics and the concepts of dissipative structures are seen to have profound implications for evolutionary theory. In particular we stress the idea that organisms possess a hierarchy of adaptive possibilities, which result from a construction of nonlinear systems each capable of structural evolution. The genome carries information capable of many "morphogeneses," as evidenced in enzyme variants. Data are presented to underline the important functional differences of these variants, and an argument put forward to support the latent multifunctional capacity of such macromolecules. A theoretical treatment of the evolution of multifunctional enzymes in the form of specialist versus generalist strategies is given, and it is shown that given considerations about volume swept, binding time, and cost of production, a generalist enzyme will do best in a dilute (or not spatially organized) system, and a specialist enzyme will do best in a dense or channelled environment. The implications of such strategies and stochastic softening of selection upon mutants are then discussed as an alternative to a strict neo-Darwinian approach to evolution.

Les auteurs interprètent de nouveaux concepts ayant trait à la thermodynamique des systèmes non à l'équilibre et les concepts relatifs aux structures dissipatives comme ayant d'importantes implications en théorie de l'évolution. Ils soulignent l'idée que les organismes présentent une hiérarchie de possibilités évolutives qui résulte de la construction de systèmes non linéaires pouvant tous subir une évolution structurale. Le génome contient de l'information autorisant bon nombre de « morphogenèses », comme le montre l'existence des variants chez les enzymes. Les auteurs présentent des données pour souligner les différences fonctionnelles importantes de ces variants et des arguments en faveur de la capacité multifonctionnelle latente de ces macromolécules. Ils traitent aussi, au niveau théorique, de l'évolution des enzymes multifonctionnelles dans le cadre de stratégies pour enzymes « spécialistes » et « généralistes » et montrent que, pour certains paramètres relatifs au volume mis en contact, au temps de fixation et aux coûts de production, une enzyme généraliste est plus efficace en système dilué (non spatialement organisé) et qu'une enzyme spécialisée est plus efficace en milieu dense ou canalisé. Ils terminent en traitant des implications de telles stratégies et de l'affaiblissement stochastique de la sélection des mutants en tant qu'alternative à une approche évolutive strictement néodarwinienne.

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The study of enzymes is one of the more important aspects of science, because it takes place at the interface of the biological and physical sciences. Advances in technology, such as X-ray diffraction methods, nuclear magnetic resonance, and fractionation techniques, have all succeeded in resolving various aspects of enzyme structure and function. These molecules play key roles in life processes, and yet details of their catalytic properties are only now being unravelled by physical chemists.

Enzymes have a tremendous capacity to organise the living organism from within: we need only cite the effects of phenylketonuria and other enzyme deficient diseases to realize the catastrophic effects of the malfunctioning of a single enzyme. Enzymes are thus portholes to our existence, allowing specific reactions to proceed and hence substances to pass in and out of the cell. Yet, the spatial and functional organization, complexity, and diversity of enzymes still makes them one of the most studied yet enigmatic group of macromolecules. Moreover, the adaptive potential of these characteristics is rarely analyzed in the context of evolution; instead the esoteric nature of the organism and the genome have captured the attention of biologists in their Panglossian search for *the* mechanisms of evolution.

In recent years, new concepts have emerged from physics and chemistry which have opened up new perspectives on our understanding of biological order and function. The ideas put forward stress the adaptability and structural evolution of which even quite simple nonlinear systems are capable. They lead to a view of living systems in which there is much more "plasticity" of behaviour than that derived from the current view in which the functions and adaptability of biological organisms are directed completely and deterministically by the in-

formation stored in the genome — information which pertains to the entire history of survival (Nicolis and Prigogine 1977).

In this paper we wish to describe briefly these new ideas, which are paralleled in many domains, in which evolutionary change, adaptability, and learning processes are found to be a very general property of nonlinear systems, and not simply an area reserved for "intelligent" beings equipped with a central nervous system. Much evidence concerning the "flexible" and highly sophisticated responses of mechanisms in the living world are now coming to light. From the intricacies of the glycolytic cycle (Goldbeter and Lefever 1972; Boiteux and Hess 1980), the extraordinary behaviour of cellular slime molds (Keller and Segel 1970), of social insects (Deneubourg et al. 1983), and of complex strategies of species within ecosystems (Allen 1985), we find that biological systems, far from being mere automatons, contain adaptive mechanisms related to stochastic processes which equip them to face the fluctuations and perturbations of the environment.

The argument is, then, that the adaptive capacity of an organism is not entirely restricted to the ability of the genetic material to undergo spontaneous mutation, and thus through selection to allow a species to "track" the environment or fill a niche, but *rather* that living systems possess throughout their whole structure layers of adaptive possibilities which result from them being constructed of nonlinear systems capable of structural evolution. In this view, the genetic material carries the information necessary to construct a complex system which itself may be capable of various "morphogeneses" in the succeeding circumstances in which it will find itself. In particular, we will apply these ideas in explaining the behaviour and functioning of the most basic macromolecules of the living world.

Dissipative Structures

The two pillars of nineteenth century science were classical physics and Darwin's theory of evolution. Not only were these two pillars disconnected, they were even in seeming contradiction with one another. For the former traced the passage of time with the destruction of existing order and the increase of entropy, while in the latter it was marked by increasing organization, diversity, and complexity. Today, however, the discovery of "dissipative structures" (Glandsdorff and Prigogine 1971) builds a firm bridge between the "living" and the "nonliving". In so doing, it offers us a new basis for the understanding of the working of biological systems. We shall therefore first briefly sketch these new ideas before going on to discuss their possible importance for understanding such processes as fundamental as enzyme reactions in the living world.

Let us turn first to classical physics and the laws of thermodynamics. These state that in an isolated system, which cannot exchange energy and matter with its surroundings, energy is conserved, and that entropy, a function of the macroscopic state of the system, will increase monotonically until it reaches a maximum. In a simple example, we may consider a closed box with a partition down the middle, to one side of which are found a number of gas molecules. When the partition is removed there is an irreversible movement of the gas molecules towards a uniform distribution within the box; the end result is thus the same regardless of the starting conditions. Boltzman's application of the probability of molecular combinations to the laws of thermodynamics only underlined the fact that in an isolated system, the formation of ordered structures must be ruled out. Thus in realizing that biological structures are highly ordered and coordinated in their functions, we must accept the need for a different approach than that provided by equilibrium statistical mechanics and thermodynamics, to describe evolutionary processes.

The key element is of course the openness of biological systems, a concept first promulgated by von Bertanlanffy. There is a flow of entropy, due to exchanges across boundaries and processes within biological systems, which was described by Clausius-Carnot as the inequality governing the variation of entropy during a time interval dt, given as

$$dS = d_eS + d_iS$$

where d_eS is the flow of entropy across boundaries and d_iS is the entropy produced as a result of internal irreversible processes. The difference between closed and open systems is thus the term d_eS; moreover, this term has no definite sign, so a situation could arise during the evolution of a system where the entropy is less than at the beginning, despite the fact that d_iS is never negative. In addition the system can be maintained in an ordered but nonequilibrium state, a situation which is akin to many events and systems at the cellular level where metabolites and enzymes react to different environments.

In a sense we can thus deduce two kinds of systems of behaviour: one which prevails in the neighbourhood of thermodynamic equilibrium, which is characterized by destruction of order, and the other, which may occur far from equilibrium and which has a specific set of nonlinear kinetic laws, that is characterized by the evolution of order. The latter is created by a flow of energy and matter from outside the system, and relies on a principle of order through fluctuations to achieve new ordered regimes. This principle is based on the expression which determines the probability of a small fluctuation in entropy around a reference state (Nicolis and Prigogine 1971):

$$P \propto \exp(\delta^2 S/2k)$$

where $\delta^2 S$ is the excess entropy. Fluctuations, which are deviations from some average state, are found throughout biological systems, and when these occur close to a transition threshold, a system may move into a new regime as certain fluctuations become amplified. The maintenance of these structures demands a flow of energy and matter from outside the system at a critical distance from equilibrium — a minimum level of dissipation — that is characterized by a particular set of nonlinear kinetics. Such configurations have been termed "dissipative structures" and are well exemplified in the chemical system described by the spiral waves of the Belousov–Zhabotinski reaction, and in the spatial dissipative structures produced by glycolyzing yeast extracts (Boiteux and Hess 1980).

A simplified model known as the Brusselator has been conceived to demonstrate the mathematical implications of dissipative structures: this model pertains to a trimolecular scheme with two intermediates, X and Y, in which

$$A \xrightarrow{k^1} X$$

$$B + X \xrightarrow{k^2} Y + D$$

$$2X + Y \xrightarrow{k^3} 3X$$

$$X \xrightarrow{k^4} E$$

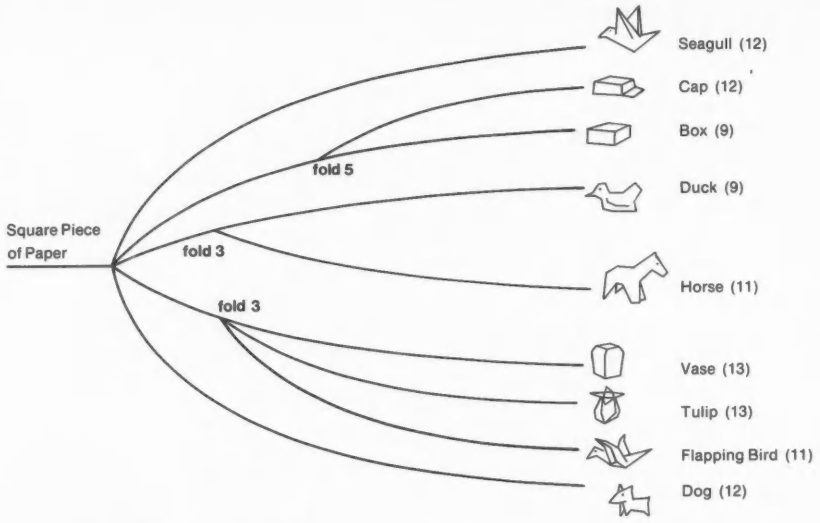

FIG. 1. A bifurcating tree of origami forms (reprinted from Allen 1985, p. 11).

where A and B are initial substrates and D and E are products. The reactions are all irreversible, and the presence of the autocatalytic step in the third reaction introduces the necessary nonlinear element to the series. We assume that E and D are removed to avoid any reverse reactions. It may also be shown (Nicolis and Auchmuty 1974) that from the kinetic equations describing the production of X and Y, given as

$$d_t\mathrm{X} = k_1\mathrm{A} + k_3\mathrm{X}^2\mathrm{Y} - k_2\mathrm{BX} - k_4\mathrm{X}$$

$$d_t\mathrm{Y} = k_2\mathrm{BX} - k_3\mathrm{X}^2\mathrm{Y},$$

and with constant diffusion coefficients and suitable boundary conditions, there exists a stationary state solution to this system given by

$$\mathrm{X}' = \frac{k}{k_4}$$

$$\mathrm{Y}' = \frac{k_2\mathrm{B}k_4}{k_3k_4\mathrm{A}}.$$

If A and B are increased, the system can move far from thermodynamic equilibrium, leading to new stable forms such as temporal oscillations or spatial structures of different kinds. When examined in *two* dimensions, dissipative structures can yield a multiplicity of forms from these reaction–diffusion equations (Nicolis and Auchmuty 1974), which are clearly nontrivial in a biological sense, because they become more and more complex as successive instabilities are traversed. Indeed the passage from one form to another via primary or secondary bifurcations may be seen as analogous to the phylogenetic tree, so often observed in evolutionary studies.

Emergence of Form

In a previous paper (Allen 1982), one of us described origami as analogous to dissipative structures. Otherwise known as the Japanese art form of paper folding, origami offers beautiful insights into the intricacies of the concepts described above, and for this reason alone it is worth reiterating this example in the context of a paper on enzymes.

From a flat sheet of paper, we can produce a variety of stable configurations simply by folding the paper in a prescribed way, a number of times (Kenneway 1980). These configurations generally relate, depending on the expertise of the practitioner, to some familiar object which we can classify. However, more important is the fact that starting from a sheet of paper with few notable landmarks a series of folds can ultimately lead to a number of forms, each of which is characterized by particular traits which have "appeared" during the folding process. We thus have an emergence of form, and essentially, qualitative, nonconservative evolution. In Fig. 1, we show that several objects must undergo the same initial folds, before a "bifurcation" point is reached, at which time the objects become differentiated, whilst others, the seagull and the dog, for example, have no folds in common with any of the other forms.

The stable configurations exist because of the tensions that have been placed upon the paper, and yet each form is physically a single piece of square paper. What is important here is that the attributes of each form are as much a realization of the folds as the order in which the folds occur. If we were to examine the sheet of paper in an unfolded state, for each form, we would be none the wiser as to its final latent form, unless we had information on the sequence of folds. This is critical because the folds or pattern of lines on the paper may be thought of as the "DNA" of the object — the "irreducible essence" of the final form, complexity thus being a reflection of the number of folds. But as shown above it is the evolution of the folds — their sequence — that is the key. What is more, the final traits emerge at critical points during the process, often without any identifiable precursor. Dissipative structures give us an example where the nonlinear interactions between molecules produce new forms and regimes which differ qualitatively in much the same way as in the origami exercise, and therefore in the kinetic equations that generate a bifurcation tree, we see for the first time a mathematics of creation.

In conclusion, the emergence of complex forms is controlled by both stochastic and deterministic forces. Close to an

unstable point, a model of the system is likely to be inadequate, as the process of evolution is subject to the environs and the chance events that may occur. Away from a bifurcation point, there is every likelihood that a simple model of the system will suffice, and indeed a particular form will persist in that mode as long as it is stable. This latter property will be conditioned by fluctuations within and outside the system, and by chance mutations. The presence of any one form is as much a manifestation of its history as the dynamics controlling its current existence.

Enzyme Structure and Function

It is clear that the most fundamental problems in enzymology have been and still are the elucidation of the structure and function of the active site and the concomitant catalytic properties of enzymes. In general terms, we understand that the enzyme-catalyzed reaction occurs within the three-dimensional confines of the active site, where a substrate is bound through a number of links, such as covalent bonding, hydrogen bonding, hydrocarbon affinity, and electrostatic attraction, as a result of conformational changes in the macrostructure of the enzyme itself. However, studies involving the description of enzymes have in turn revealed a surprising level of variation. This variation can be seen at many levels and in many ways, including the amino acid sequence of an enzyme, its stereochemistry, and its electrophoretic mobility.

For example, it has been shown that the amino acid sequence of an enzyme, whilst obviously critical in some regions, can be subject to large changes without functional impediment. The cytochrome *c* group, which is characterized by its heme connections and absorption spectra, contains members whose chain lengths are 102–112 in eukaryotic organisms, 85–124 in purple nonsulfer bacteria, 134 in *Paracoccus*, 82 in *Pseudomonas*, and 86 in *Chlorobium* (Dickerson and Takano 1978). These cytochrome molecules all have the same general folding pattern and heme environment, and differ mainly in the addition and deletion of loops of chain on the surface of the molecule. On the other hand, it is interesting to note that in certain members, c_2, the two physiological functions of the family, photosynthesis and respiration, are combined. Moreover, cytochrome *c* operates in both oxidative and reductive environments, and in nonmitochondrial and mitochondrial forms. This family tree, therefore, reflects significant functional evolutionary changes, not unlike the origami tree in Fig. 1, and supports the fact that homologous enzymes, whilst retaining essentially identical active sites, may differ significantly in their catalytic and/or substrate-binding properties.

The stereochemistry of enzyme-catalyzed reactions can also be looked at from an evolutionary viewpoint. For example, we can examine the NAD(P)-linked dehydrogenases with regard to the stereochemistry of hydrogen transfer to their coenzyme. Initial work by Westheimer and others (Westheimer et al. 1951; Loewus et al. 1955) indicated that the transferred hydrogen is located at the C-4 position of the nicotinamide ring, and in hydrogenases classified as A type it is tranferred to the pro(R) position, whilst in B types it is transferred to the pro(S) position. In addition, several rules have been proposed (Krakow et al. 1963; Alizade and Brendel 1975) correlating substrate type and the stereochemistry of the transfer of hydrogen. One of these states that "most dehydrogenases which are not specific for either NAD or NADP but can transfer hydrogen to both coenzymes are of the B type" (Alizade and Brendel 1975). The binding sites of these dehydrogenases are also reported to be similar, despite the stereospecificity of hydrogen transfer; thus, it is interesting to note that alcohol dehydrogenase NAD(P) (EC 1.1.1.71) is an A type enzyme for both NAD and NADP (Rossman et al. 1974). It is postulated (Rossman et al. 1974) that an original NAD-specific enzyme retained its original stereochemistry of hydrogen transfer, but evolved to a form without coenzyme specificity. In another case, D-glucose-6-phosphate dehydrogenase (NADP) and D-glucose dehydrogenase (NAD) are classified as type B; but it has been shown that the latter enzyme can also metabolize glucose-6-phosphate using NADP. These two enzymes may thus have evolved from either form (Thompson and Carper 1970).

Evidence of the same phenomenon described above can also be seen within a species where electrophoretically alternate forms of an enzyme, isozymes, occur. The traditional view is that these are due to either the existence of multiple gene loci coding for distinct versions of the enzyme protein, for example the mitochondrial and cytoplasmic isozymes of malate dehydrogenase (EC 1.1.1.37), or to the occurrence of multiple alleles at a single gene locus. In the case of lactate dehydrogenase (EC 1.1.1.27), both explanations hold. The enzyme occurs as a tetramer made up of at least two types of polypeptide chain, one of which is predominant in the heart and one in the muscle. In their pure forms, i.e. all heart (B_4) or all muscle (A_4) type, these two isozymes have very different kinetic properties, with the intermediate forms showing intermediate properties. Generally the quantitative distribution of the five forms (A_4, A_3B, A_2B_2, AB_3, B_4) is binomial, suggesting that the A and B subunits associate randomly, although this is not always the case; for example in herring (*Clupea harengus*) the asymmetric heteropolymers are usually absent. In addition, many species of fish exhibit allelic variation at one or more of the LDH loci.

Further advances in our understanding of the functional aspects of enzymes have also been forthcoming during the past decade, and indeed there is some feeling that "little progress toward the understanding of protein reactions is possible so long as proteins are relegated in our imaginations to passive structural roles and pure catalysis" (Lumry and Biltonen 1969). We know that in general terms an enzyme reduces the activation free energy by dividing up the reaction into discrete steps, thus reducing the free-energy barrier at each one: but just how this process is achieved is still not clear. Some of the most important work examines this problem with respect to the principle of order through fluctuations discovered in the study of dissipative structures, and in an excellent review by Welch et al. (1982), we see the enzyme as a dynamic entity, which in the "cytosociological" habitat (Welch and Keleti 1981) of the cell effects catalysis by undergoing conformational fluctuations which have a well-defined time correlation in the order of nanoseconds (Careri et al. 1979). Thus, the three-dimensional form arrived at from X-ray studies is only an *average* form of the atomic displacements within the macromolecule. Careri and his co-workers have stressed the importance of the temporal sequences of conformational changes to produce thermal fluctuations, as a result of solvent interactions, which in turn provide the thermal energy for the free-energy events in the active site. The changes in conformation are related to charge density fluctuations, and it has been postulated that the large size of enzyme molecules, relative to the active site, is a reflection of

TABLE 1. Activity levels for the three lactate dehydrogenase phenotypes observed in *Pollachius virens* given in units per litre of each enzyme.

	Heterozygote	Homozygote	Alternate homozygote
Alanine	800	979	528
Glutamic acid	5 005	2 882	2 134
Lactic acid	17 336	17 457	18 612

the importance of the rest of the macromolecule to provide not only the free energy to allow the enzyme-bound reactant(s) to proceed to the transitional complex, but also to allow the enzyme to apparently act in a "deterministic" fashion in an otherwise chaotic solvent medium.

In the simple case, then, we should expect enzymes to act in accordance with the concept of substrate and functional specificity, yet we know that there are enzymes which react with a range of substrates and/or display an ability to catalyze more than one type of reaction. For example, we know that lactate dehydrogenase can dehydrogenate not only lactic acid but also glyceric acid, α-aminolactic, and β-haloactic acids (Anonymous 1973) and can hydrogenate a number of α-ketoacids besides pyruvate, and also diketoacids. Moerover, in an electrophoretic study of LDH in gadoids, it was found that the activity levels associated with alternate substrates such as alanine and glutamic acid were different in individuals which were homozygous, heterozygous, and homozygous for an alternate allele at one of the lactate dehydrogenase loci (Table 1). Thus, it is conceivable that the alternate forms of the LDH molecule, whilst showing little change in their catalytic properties with lactic acid, are more sensitive when exposed to other less commonly used substrates. It should of course be borne in mind that we are analyzing the enzyme *in vitro*, out with its usual cytosociological environment, but in fact we may be seeing an unfulfilled potential in an otherwise single functioning enzyme.

More examples of these phenomena are forthcoming; in the case of the dehydrogenases, for example, alcohol dehydrogenase (EC 1.1.1.1) is highly specific for NAD but can react with a number of alcohols. There are also some hydrolyzing enzymes, such as esterases, phosphatases, and peptidases, which can catalyze reactions with an array of carboxylic esters, phosphate esters, and peptides, respectively, although contained within these groups there are some enzymes which are highly specific. In another case, xanthine oxidase (EC 1.2.3.2) catalyses the decomposition of hypoxanthine and xanthine, but is also generally unspecific; as an electron donor it reacts with aldehydes, purines, pteridines, pyrimidines, azapurines, and other heterocyclic compounds, plus the oxidation of purine-antimetabolites (Duggan and Titus 1959). Similarly, aldehyde oxidase (EC 1.2.3.1) also oxidizes quinoline and pyridine derivatives. There are also dual function enzymes which act on a substrate in different ways, for example malate and isocitrate dehydrogenases (EC 1.1.1.38, EC 1.1.1.40, and EC 1.1.1.42), which reduce or decarboxylate their corresponding oxoacids.

How then do these enzymes provide the organism with such flexibility when their actions are linked with a set of spatio-temporal coordinates? One answer may lie in the number of active sites that an enzyme possesses; for example the binding of nucleotides to glutamate dehydrogenase ($NAD(P)^+$) (EC 1.4.1.3) is regulated by polymerization of the enzyme. However, the "purposive" behaviour of enzymes will also reflect the attractivity of certain substrates, and it is conceivable that initial binding or contact may initiate a set sequence of conformational changes; the range of reactions would be potentiated by atomic fluctuations in the enzyme allowing the macromolecule to move through various sequences in a fashion akin to the origami example.

In this sense we can see the real adaptive role that enzymes have to play, as they form a critical link between the heterogeneous and variable intracellular microenvironment, and the spatio-temporal organization and deterministic responses at other levels in the organism. Moreover, the range of activities that such a multiplicity of forms routinely generates is indicative of the potential ability that these macromolecules have to adapt the complex structures produced by the genome and enable them to evolve into other forms, in response to changes in the environment. Indeed we have seen the creation of spatial dissipative structures from a homogeneous biochemical system, in the spatial patterns of reduced pyridine nucleotide produced by glycolyzing yeast extract (Boiteux and Hess 1980).

In other words, instead of functioning as a genetically determined machine in a predictable environment, we have instead a genome which produces molecules with a flexible response to a chaotic environment. This in turn implies a nondependence on gene amplification to produce tissue specificity in enzyme loci, for we know that it requires only a very weak field to orient a bifurcation in the folding pattern, and hence potentially produce an alternate form of a functionally similar enzyme.

Evolution of Variation in Enzymes: Specialist and Generalist Strategies

Accepting that enzymes can and do play a critical role in adapting the organism to its environment, it is important for us to examine enzymes as if they have some control over that role. One of the most fundamental questions concerning enzymes in this respect is whether to produce a number of highly efficient specialized enzymes, or a fewer number of more general enzymes. In the light of the experimental evidence cited above, there are clearly examples of both types, and so we should like to present a simple theoretical consideration of this problem.

Let us suppose that the energetic "costs" of an organism are proportional to the number of enzymes that have to be produced and maintained, in order to obtain the necessary reaction rates with all the various substrates provided under normal conditions. We can now examine how this cost might be minimized under different circumstances: in general

$$E + S \xrightarrow{\alpha} E^*$$

$$E^* \xrightarrow{1/\tau} E + P$$

where E is the enzyme, S is the substrate, P is the product, E* is the catalytic intermediate, α is the volume "swept" per unit time (cross section × velocity), and τ is the binding time. The kinetic equations for this scheme are as follows, assuming that the density of substrate and product remain relatively constant during the time of binding. Therefore ($e*$, e, s = densities):

$$\frac{de}{dt} = \frac{e*}{\tau} - \alpha e s$$

$$\frac{de*}{dt} = \alpha e s - \frac{e*}{\tau}.$$

Therefore:

$$\frac{de}{dt} + \frac{de*}{dt} = 0.$$

Let $e + e* = e_0$ (total enzyme density). If we assume that $de*/dt \leftrightarrow de/dt$ is very rapid compared with the overall reaction, then:

$$\frac{de}{dt} = 0 = \alpha e s - \frac{(e_0 - e)}{\tau}$$

and hence

$$e = e_0 \frac{1}{(1 + \alpha\tau s)}.$$

From this the overall reaction rate is

$$-\frac{dS}{dt} = \frac{dP}{dt} = e\alpha s = \frac{e_0 \alpha s}{1 + \alpha\tau s}.$$

Now let us consider the problem when more than one substrate must be handled, two for example. There are two possibilities. Either we will have two specific enzymes, one for each substrate, or a single, dual purpose enzyme. Let us consider the simplest case when the "benefit" of the two reactions is simply viewed as being additive. Then the total reaction rate is for two specific enzymes:

$$\frac{dP_1}{dt} + \frac{dP_2}{dt} = \frac{\alpha_1 e_1 s_1}{1 + \alpha_1 \tau_1 s_1} + \frac{\alpha_2 e_2 s_2}{1 + \alpha_2 \tau_2 s_2}$$

and for one dual purpose enzyme:

$$= \frac{\alpha e (s_1 + s_2)}{1 + \alpha\tau(s_1 + s_2)}.$$

We know also that a "specialist" enzyme will in general be more "effective" than an all purpose one, so let

$$\alpha_1 = \alpha_2 = (1 + \epsilon)\alpha \quad (\epsilon > 0)$$

$$\alpha_1 = \alpha_2 = (1 - \eta)\tau \quad (\eta > 0).$$

Then, comparing the two possibilities, and for simplicity putting $s_1 = s_2 = s$, then

$$\underbrace{\frac{(1 + \epsilon)\alpha s (e_1 + e_2)}{1 + (1 + \epsilon)(1 - \eta)\alpha\tau s}}_{\text{(2 specialists)}} \overset{?}{\gtrless} \underbrace{\frac{2\alpha s e}{1 + 2\alpha\tau}}_{\text{(1 generalist)}}.$$

The "cost" of each strategy is $c(e_1 + e_2)$ for two specialists and ce for the generalist. Thus, the choice simply depends on

$$\frac{(1 + \epsilon)\, s}{1 + (1 + \epsilon)(1 - \eta)\alpha\tau\, s} \overset{?}{\gtrless} \frac{2\alpha s}{1 + 2\alpha\tau}.$$

Situation I

When the system is very dilute in substrate S, then $\alpha\tau s \ll 1$. This reduces to

$$(1 + \epsilon)\alpha s \gtrless 2\,\alpha s.$$

Now, only if each specialist enzyme were twice as effective at attracting its substrate would it be advantageous to be a specialist. In general, this is impossible at the same cost! So when the system is dilute (or not spatially organized), then the generalist is best.

Situation II

When the system is not dilute in substrates, then $\alpha\tau s \gg 1$. Then,

$$\frac{(1 + \epsilon)\alpha s}{(1 + \epsilon)(1 - \eta)\alpha\tau s} \overset{?}{\gtrless} \frac{2\alpha s}{2\alpha\tau s}.$$

Therefore:

$$\frac{1}{(1 - \eta)\tau} \overset{?}{\gtrless} \frac{1}{\tau} \qquad \frac{1}{(1 - \eta)} \gtrless 1.$$

But $\eta > 0$; therefore, the left-hand side is greater, and hence more advantageous. For conditions where the substrate is very dense, or is *channelled* to the enzymes, then the specialist solution is advantageous.

Additionally, in the first situation $\alpha\tau s \ll 1$; thus, the binding time is very unimportant, and only the "attractivity" α will matter, whereas in the second situation, the roles are inverted and only the binding time matters. In an evolutionary perspective, we may envisage a first phase in which the "channelling" of substrate is improved, followed by a second in which the rate of reaction itself is increased.

If enzymes possess such organizational and adaptive potential, we must ask ourselves the fundamental question of how the various forms initially appeared and survived extinction. The majority of existing models clearly adhere to the strictly neo-Darwinian deterministic approach, in which a mutant must be initially advantageous to succeed. However, in a paper by Allen and Ebeling (1983), it was shown that mutations could survive for considerable periods, even if they only occurred initially in very small numbers. The approach they used to describe the dynamic interactions of various population types was a basic stochastic process, which showed that a system could "experiment" with seemingly nonadvantageous mutations for a significant time. Thus, evolution could be preceded by some probing of the surrounding "landscape" before any commitment was made. Such stochastic "softening" of selection would clearly be an important step in the eventual emergence of alternate forms, viz. functionally different but related enzymes, for it would give the organism some flexibility in responding to its environment, which was previously only afforded by gene duplication; it would also be a critical step in replacing the strictly neo-Darwinian model of evolution.

Conclusions

Today our understanding of the natural world has grown to incorporate some elements of the complexity that surrounds us. Through nonequilibrium thermodynamics, and the concepts of dissipative structures, it is now possible to reexamine some of the cornerstones of evolutionary theory, and arrive at a significantly different viewpoint. In applying these concepts to the structural and functional aspects of enzymes, it is clear that these macromolecules have at their disposal a mechanism, whereby they could evolve different structural forms from initially the same genomic material, and could also transcend functional constraints through fluctuations experienced at critical — bifurcation — points. Whilst the experimental evidence for multifunctionalism is sparse, we do know that such enzymes exist, and in a theoretical consideration, it is possible to arrive at a set of conditions, viz. in a system that is not spatially organized or in which the substrates are very dilute, where it would be advantageous to have a multifunctional

enzyme rather than one which was specific. To allow such mutant forms to originally survive, we suggest that a stochastic approach is more plausible than the deterministic stand which is characteristic of neo-Darwinism because through stochastic softening of selection, seemingly nonadvantageous forms are maintained in the population for significant periods of time. It is obvious that there is a large gap between the theoretical considerations and the experimental evidence that has accrued to date, but this is a symptom of the complexity of the systems with which we are dealing. However, it is clear that to make any progress in our understanding of the evolution of enzymes and organisms, we must deal with the entire range of dynamic potentialities, rather than be content with the formulations to date that have been based on average and static descriptions.

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Artificial Selection on Growth and Correlated Selection on Competitive Behaviour in Fish

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Doyle, R. W., and A. J. Talbot. 1986. Artificial selection on growth and correlated selection on competitive behaviour in fish. Can. J. Fish. Aquat. Sci. 43: 1059–1064.

There is widespread concern that artificial selection for rapid growth will indirectly select for aggressive fish, thus nullifying genetic stock improvement programs based on selection. We show that this unfortunate result is highly unlikely. The argument is based on a game-theoretic analysis of two general features of fish competition: (1) the growth of some individuals is often suppressed even when food is abundant and (2) the behaviour of a fish towards other members of a population depends on its relative size. The commonsense view that growth rate selection favours aggressive fish is shown to be correct only when aggressive behaviour is independent of relative size and resources are severely limited. In experimental and aquaculture environments, growth rate selection is shown to favour a decrease in the value of both aggressive and submissive behaviour, i.e. fish which ignore each other. Artificial selection for rapid growth will therefore indirectly select for tameness, not aggression.

Bon nombre de spécialistes s'inquiètent du fait que la sélection artificielle visant à favoriser la croissance rapide puisse indirectement favoriser la sélection de poissons agressifs, ce qui rendrait inutiles tous les programmes d'amélioration génétique des stocks basés sur la sélection. Les auteurs montrent qu'il est très peu probable qu'un tel résultat soit obtenu. L'hypothèse à la base de cette conséquence nuisible repose sur une analyse par théorie des jeux de deux caractères généraux de la compétition chez les poissons : (1) la croissance de certains individus est souvent supprimée, même en présence d'une nourriture abondante et (2) le comportement d'un poisson envers ses congénères d'une même population est fonction de sa taille relative. Les auteurs montrent que l'hypothèse générale voulant que la sélection pour le taux de croissance favorise les poissons agressifs ne s'avère correcte que lorsque le comportement d'agression est indépendant de la taille relative et que les ressources sont fortement limitées. En milieux expérimentaux ou aquicoles, la sélection pour le taux de croissance favorise une diminution de la valeur des comportements d'agression et de soumission : elle favorise les poissons qui s'ignorent. La sélection artificielle visant la croissance rapide favorisera donc indirectement celle de poissons domestiqués et non celle de poissons agressifs.

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Intra-population competition among fish is potentially a serious problem for aquaculture geneticists. If the biggest fish in a generation are merely those that through behavioural dominance capture most of the food, superior growth may bear little relation to genetic variation in assimilative capacity or physiological growth rate (Purdom 1974; Weatherly 1976; Malecha 1977; Hedgecock et al. 1982). This possibility gives rise to the widespread fear that selection of faster growing fish will produce fish that are merely more competitive, not more productive. Artificial selection for improved growth will then produce an undesirable shift towards higher levels of aggression in domesticated fish. Kinghorn (1983) expressed this concern in a recent review of the current state of aquaculture genetics: "... in a competitive environment, genetic variation in growth can have a component due to competing ability. As genetic improvement in competing ability is inevitably paralleled by a similar improvement in the mean competing ability of all contemporaries, there can be no net improvement [in growth]." If this view is correct, the desire of aquaculture geneticists to duplicate the successes of animal breeders may never be realized.

There can be no doubt that competitive interaction among individuals is a major source of growth rate variation under the usual culture conditions (Purdom 1974; Moav 1979; Moav and Wohlfarth 1974; Noakes 1978; Kinghorn 1983). The principal effect of competition is to increase the variance and skewness of the size-at-age distribution (Wilbur and Collins 1973; Purdom 1974; Brett 1979). Increased coefficients of variation when fish are reared in groups rather than in isolation have been observed in plaice, flounder, and their hybrids (Purdom 1974), trout (*Salmo trutta*) (Heland 1971a, 1971b), medaka (*Oryzias latipes*) (Magnuson 1962), sunfish, (*Elassoma evergladei*) (Rubenstein 1981), and carp (Nakamura and Kasahara 1961). The close relationship between the heterogeneity of growth rates and social dominance hierarchies based on size and/or territoriality has been noted in many fish species (Noakes 1978). Other aquatic poikilotherms seem to react to group rearing in a similar way, for example amphibian tadpoles (Wilbur and Collins 1973; Rose 1960; Steinwascher 1978), prawn (*Macrobrachium rosenbergii*) (Malecha 1980), and lobsters (*Homarus americanus*) (Aiken 1977; Nelson et al. 1980).

An essential feature of competitive rearing is that the suppressive effect is unequally distributed among the members of the population. As is true of plants grown under competitive conditions (Harper 1977), the most abundant individual in a fish population is often a "suppressed weakling." Begon (1982) observed that it is as if the smaller individuals perceive the population density as being much higher than it is (on average),

while the larger individuals perceive the density as being much lower than it is.

Rubenstein (1981), for example, demonstrated that when pygmy sunfish compete for food, the growth of the best competitors was not diminished nearly as much as the average fish at high population densities. This was also true of the *Rana pipiens* tadpole cultures of Rose (1960), in which the size of the largest individuals in all similarly sized containers was the same, regardless of the number of small individuals in the same container. The decrease in average growth rate with increasing population density was not because all individuals were stunted but because more were. In a cage-culture experiment with the grouper *Epinephalus tauvina*, Chua and Teng (1978) observed that when feeding frequency varied between once every 5 d and twice per day, the final size of the largest measured fish varied by only 8% but that of the smallest fish (and also the modal fish) varied by about 40%. Later experiments (Chua and Teng 1979) showed that when density was increased from 15 to 120 fish/m^3 with a constant feeding frequency, the corresponding figures were 4 and 40%.

Resource-Independent Competitive Effects

The simplest view of the effects of competition on fish is that allocation of a resource (food) controls relative growth rates. A disproportionate amount of food goes to superior competitors which in consequence grow faster. We will call this variance-generating process "resource-dependent competition," because it requires that a fish outcompete another by reducing the amount or quality of food available to it.

If the probability of success in a competitive encounter between fish depends directly or indirectly on size, as is usually the case when size variation is appreciable (Noakes 1978), the distribution of resource exploitation will amplify differences in size and growth rate, leading to an increased coefficient of variation. When size differences do not exist or are very small, dominance hierarchies may not be correlated with size differences at first, but become so later as dominance is translated into superior net energy gain (Yamagishi 1962). Reduced growth and increased mortality of unsuccessful competitors may be attributable both to starvation and the metabolic costs of competition for territories which provide access to food (Miller 1958; Symons 1971).

In natural populations, unequal resource availability may well be the usual cause of growth rate variation. Lomnicki and Ombach (1984) suggested that such variation is essential to the stability of single-species populations in nature. In managed environments, however, monopolization of a limiting food resource by competitively superior individuals is *not required* for competitive effects to be seen. Many experiments have shown that competitive effects can persist even when no resource-dependent influence is possible. Nagoshi (1967) found that the growth of behaviourally subordinate *Poecilia reticulata* was suppressed by the dominants over a large range of food supply. Allee et al. (1948) observed that in their laboratory experiments, behaviourally dominant individual green sunfish (*Lepomis cyanellus*) ate more and grew faster than subordinate individuals, although the fish were well fed and close observation failed to detect any priority of access to food. Purdom (1974) noted that the effect of dominance hierarchies on the growth rate variance of his plaice/flounder hybrids could not be avoided merely by feeding to excess. Molander and Molander-Swedmark (1957) demonstrated the same thing in 0-group plaice (*Pleuronectes platessa*). Koebele (1985) found that isolated *Tilapia zillii* in visual contact with each other tended to grow at different rates, with the "visually dominant" fish consistently consuming their entire ration, while the "visually subordinate" fish did not. All of the fish were provided with the same amount of food in his experiment.

Working with Atlantic salmon (*Salmo salar*), Li and Brocksen (1977) observed that variance in metabolic rate, activity, and growth could in part be attributed to starvation of submissive individuals despite excess food, coupled with distraction of the dominants by agonistic activities during feeding periods. Subordinate salmon and trout tend to reduce their feeding and cower under rocks in experimental raceways even when not being threatened and in the presence of food sufficient to satiate all the fish (Symons 1971; Heland 1971a, 1971b). Subordinate lobsters exhibit similar behaviour (Nelson et al. 1980). Jobling (1985) has shown that the effect of competition on growth rate variance in Arctic char (*Salvelinus alpinus*) does not depend on territoriality or the formation of direct linear dominance hierarchies.

Waterborne growth inhibitors or "crowding factors" (Styczynska-Jurnewicz 1979) provide the clearest instances of resource limitation being unnecessary to the increased variance associated with group rearing. The existence of chemical factors which inhibit the growth of some individuals despite excess food has been repeatedly demonstrated in fish (Yu and Perlmutter 1970; Pfuderer et al. 1974), crustaceans (Nelson et al. 1980; Malecha 1980), and the aquatic stages of amphibians (Rose 1960; Richards 1958; West 1960).

It is assumed by many authors that success in resource-dependent competition is necessary for an individual to grow at the maximal rate (e.g. Kinghorn 1983; Wilbur and Collins 1973; Weatherly 1972, 1976; Purdom 1974; Chevassus 1982). This is the basis of the fear that growth rate selection will produce more aggressive, not faster growing, fish. The same assumption of resource limitation is usually made in discussions of the effect of competition on quantitative genetic parameters (e.g. Falconer 1981, p. 146; Moav and Wohlfarth 1974). However, the experiments mentioned above indicate that competitive interactions can affect fish populations even in properly managed environments where resources are abundant. Success in competition may be irrelevant to obtaining maximal energy in an environment managed in this way where both "victor" and "vanquished" in competitive encounters achieve less than the maximal reward. This point is crucial to the game-theoretic arguments presented below.

Size-Dependent (Contingent) Behaviour

Another aspect of competitive behaviour must be incorporated in analyses of fish competition, namely that the aggressiveness or competitive ability of an individual has a variable expression. It is a common observation that individual behaviour can be altered by changing the size, or sometimes territorial status, of a fish relative to its competitors.

Nakamura and Kashahara (1957, 1961), demonstrated that the appearance of unusually large carp in a population of even-aged juveniles can be suppressed by the addition of somewhat larger individuals (which then become unusually large them-

selves) and that the suppression is reversible. Chevassus (1982) suggested that increasing coefficient of variation is a widespread response to "grading" an initially heterogenous group of fish into homogenous size classes. He interpreted this as release of repression of some but not all of the smaller fish following removal of the larger dominants. Gunnes (1976) reported that after grading Atlantic salmon on the basis of size, the coefficient of variation of fish which had been smaller than the modal size increased greatly, even though food had been fed to excess at all times.

When fish grown in groups are subsequently isolated as individuals, the coefficient of variation drops sharply as the fish which are smaller but have a higher size-specific growth rate catch up (Purdom 1974). Rose (1960) and Richards (1958) found that stunted individual tadpoles of the frog *Rana pipiens* which are removed from the influence of larger animals grow well; if not removed they stop growing and die. Molander and Molander-Swedmark (1957) observed that when uniform groups of suppressed plaice were moved to an empty aquarium away from larger individuals, inequalities in activity and food consumption gradually reestablished themselves and resulted in unequal growth rates.

In summary, the experiments reviewed in the preceding sections indicate that models of competition among fish should take two aspects of fish behaviour into account: (1) that the effect of competition on growth rate variation can often be observed when food is abundant and (2) that the most usual competitive behaviour is to be dominant and aggressive when large and subordinate and submissive when small.

Game-Theoretic Analysis of Competition

We proceed by classifying the behaviour phenotypes exhibited by fish during contests over food into five categories. It is supposed, initially, that some fish may always be aggressive, some always submissive, and some may switch behaviour depending on their relative size. Among those that switch, it is supposed that one subcategory may be more aggressive and less submissive than the standard type during interactions, while another subcategory may be both less aggressive and less submissive. Later on in the analysis the classification of phenotypes into five categories and subcategories is relaxed. For convenience in the discussion the five phenotypes have been given names, as follows.

A, pure Aggressor, for which the value of competitive success (i.e. the energy value of a food item after subtracting the cost of display, pursuit, etc.) is V and the penalty for defeat in combat (i.e. damage repair) is P. Pure Aggressors threaten, and if their opponent fails to retreat attack, regardless of size; if a fight occurs the larger animal wins with probability q.

S, pure Submitter, for which the payoff for victory is V and payoff for retreat is 0. Pure Submitters are victorious over smaller, nonaggressive animals with probability m but retreat from attackers of any size.

C, Contingent, which behave as Aggressors when large and Submitters when small. A contest between a large animal in aggressive mode and a smaller animal in submissive mode always results in the larger fish getting the food.

U, Uninvolved, a less-aggressive variant of Contingent which retreat the minimum necessary distance to avoid damage when attacked, otherwise ignore other animals. The value of a food item for a successful Uninvolved animal is $V + v$, greater than V because U does not expend energy in threat or attack; the payoff for retreat is v, greater than 0 if U conserves energy by minimizing unnecessary display and submissive behaviour.

AC, Aggressive–Contingent, a more aggressive variant of the Contingent phenotype. AC animals fight larger A and C phenotypes with probability g, and the probability of their winning such encounters is $(1 - q)$.

The behaviour phenotypes are a modification of the extended "Hawk/Dove/Assessor Game" analysed by Maynard Smith and others (e.g. Maynard Smith 1982). Because of small but important changes in definitions, we have tried to avoid confusion with the usual terminology by adopting new names for the behaviour of fish, but the close similarity of Maynard Smith's "Hawk, Dove, Assessor" to our "pure Aggressor, pure Submitter, Contingent" is obvious.

In the situation envisaged here the contests are assumed to be asymmetric (larger versus smaller), animals are assumed to recognize this asymmetry, and the payoffs and costs of combat are correlated with the asymmetry. These assumptions are not necessarily valid in practice. Unusually intense agonistic interactions between fish of approximately the same size have often been noted, which suggests that fish can be unsure or mistaken in their mutual appraisal. Some of the formal properties of such "incomplete information" contests have been studied both strategically (e.g. Hammerstein and Parker 1982) and genetically (Bomze et al. 1983).

In fish, as in many other animals, actual combat may be averted or diminished by the flight or submission of one of the contestants after a reciprocal display. The winners and losers in these ritualized contests are often decided by asymmetries in size or possession of territory exhibited during the display. Both contestants presumably incur some costs in each encounter, including loss of feeding time, metabolic expenditure of energy associated with display, flight and pursuit, damage sustained during "nipping", etc. The winner gains the contested resource and his costs in doing so may be either higher or lower than the costs to the loser (other than the loss of the resource itself). If a ritualized contest escalates to an actual fight, the cost to both contestants may be substantially higher.

It is conventional in evolutionary game theory to describe agonistic encounters by a "payoff matrix", E, in which the elements $E(y,x)$ represent the expected increment in fitness obtained by an individual exhibiting behaviour y in an encounter with an animal exhibiting behaviour x. In the present paper we assume repeated, random encounters between pairs of fish in the population. Each encounter is a brief contest over an item of food which is immediately consumed and ultimately contributes to increased fitness. The game-theoretic arguments require that competitive behaviour be expressed in terms of its effect on relative fitness. Since artificial selection for rapid growth will choose large animals as breeders whether or not they are also breeding naturally in the population, we can unambiguously calibrate the costs and rewards of a contested food item in terms of contribution to relative size.

The payoff matrix for a mixture of A, S, and C phenotypes is

	A	S	C
A	$(V - P)/2$	V	$[(2 - q)V - qP]/2$
S	0	$V/2$	$mV/2$
C	$[qV - (1 - q)P]/2$	$(2 - m)V/2$	$V/2$

and for AC, C, and U phenotypes is

	AC	C	U
AC	$(V - gP)/2$	$\{V(2 - m) - g[q(V + P) - mV]\}/2$	$(2 - m)V/2$
C	$[V + g(q - 1)(V + P)]/2$	$V/2$	$(2 - m)V/2$
U	$(mV + 2v)/2$	$(mV + 2v)/2$	$(V + 2v)/2$

By convention, the payoffs in the matrix represent the payoffs and costs accrued by the animal on the vertical axis when it encounters an animal on the horizontal axis. In the simple case in which each animal can adopt only one type of behaviour (pure strategy), the behaviour $x*$ which becomes evolutionarily stable (if any) is that for which the following conditions hold (Bishop and Cannings 1976):

$$E(x*, x*) \geq E(x, x*)$$

and, if equality holds,

$$E(x*, x) > E(x, x) \text{ for all } x \neq x*$$

where $x*$ is the evolutionarily stable strategy and x is any other rare ("mutant") strategy.

In principle, any of the behaviours, either singly or in combination, might be adopted by fish of any size. Mixtures of two or more of the four possible pure strategies may be stable, and populations may also evolve towards a mixture which is not stable in the Maynard Smith sense (Zeeman 1981). While the complete analysis of this five-phenotype matrix would be very complex, it seems unnecessary to examine all possible combinations of the elements of the behaviour set in artificial environments. In particular, we can safely exclude combinations in which individual animals are always aggressive or submissive, or alternate between these behaviour modes according to some stochastic rule. Switching does occur, but the experimental evidence overwhelmingly indicates that switching is based on relative size. The empirically plausible strategy set encompasses three main variations on the same behaviour pattern: Aggressive–Contingent, Contingent, and Uninvolved.

The A–S–C matrix has pure C as an evolutionarily stable equilibrium when

$$V < qP/(1 - q).$$

The likelihood of Contingent behaviour being stable is increased when the payoff for obtaining any single food item is low, the penalty for losing a fight is high, and/or the outcome of a fight is strongly dependent on the relative sizes of the contestants. We have not investigated possible stable mixed strategies because mixtures of Aggressors and Submitters can be excluded on empirical grounds.

The AC–C–U matrix has U as a pure strategy when

$$V < 2v/(1 - m).$$

Uninvolved behaviour is stable if the payoff for a food item is low and/or the energy saved by not displaying and retreating unnecessarily is high. As in the A–S–C matrix the advantage of U also depends on the correlation between size and success. The effect of m on the ESS raises the possibility that selection for Uninvolved fish might be increased in environments where the size–success correlation in noncombatitive contests is high, e.g. in clear water (visual appraisal) or well-mixed water (chemical suppressors). Aggressive–Contingent and Contingent animals may increase their energy-wasting interactions when they can see farther.

These inequalities define the indirect selection problem which Purdom (1974), Kinghorn (1983), and others suggested may interfere with selective stock improvement. The evolution of a population of pure Aggressors could indeed result when feeding is competitive and food is limited so that the payoff for each contest is high. In such circumstances the only way a fish can grow large enough to be selected is by taking food from other fish. We believe, however, that in managed environments this is unlikely because the energy reward for aggression can be reduced by suitable feeding procedures. The Uninvolved phenotype is superior in environments in which competition does not cause resource limitation directly (i.e. because the more successful fish can monopolize a growth-limiting resource).

Selection Direction and Intensity

The discrete-behaviour models considered thus far have the limitation that behaviour is chosen from a finite set of possibilities and is "all-or-none." We note, however, that the Aggressive–Contingent and Uninvolved phenotypes are actually just subcategories of Contingent. It is more useful for purposes of genetic analysis to consider a model which permits a continuous gradation of behaviour, and then investigate the direction and rate of evolution along this gradient. The AC–C–U matrix is continuous in the sense that an infinite number of mixtures of the pure types is theoretically possible, but not in the sense that an individual can adjust the intensity of its behaviour on a continuous scale or that intensity can vary continuously among individuals in the population.

There are two continuous measures of "aggression" available in the present analysis, namely g, the probability that an animal will engage in combat even when relatively small, and v, the amount of energy lost in unnecessary display and retreat.

To calculate the selection intensities on these continuous traits, we let the proportion of the three phenotype strategies (say AC, C, U) in a mixed population be represented by the vector $a = (a_1, a_2, a_3)$. The mean energy gain per encounter in the population is

$$\bar{W} = (a_1, a_2, a_3) \begin{pmatrix} E(\text{AC,AC}) & E(\text{AC,C}) & E(\text{AC,U}) \\ E(\text{C,AC}) & E(\text{C,C}) & E(\text{C,U}) \\ E(\text{U,AC}) & E(\text{U,C}) & E(\text{U,U}) \end{pmatrix} \begin{pmatrix} a_1 \\ a_2 \\ a_3 \end{pmatrix}$$

or

$$\bar{W} = aEa.$$

The selection intensity on a trait X is the weighted mean of the selection intensities on each of the phenotypes in which it occurs, i.e. the gradient of fitness on X:

$$i_x = \left(\frac{\partial \bar{W}}{\partial X}\right)_a.$$

In a population of pure AC or an AC−U mixture, $a_1 \gg a_2$ and

$$i_g = a_1^2 \left(\frac{\partial E(\mathrm{AC}, \mathrm{AC})}{\partial g} \right) = \frac{-P}{2}.$$

Selection is negative and increases as the penalty for combat increases.

In a population of *almost* pure C phenotypes, $a_2 \gg a_1$ and

$$i_g = \frac{a_1 a_2}{2} \left(\frac{\partial E(\mathrm{AC}, \mathrm{C}) + \partial E(\mathrm{C}, \mathrm{AC}))}{\partial g} \right) = \frac{-(1 - m)V - P}{2}.$$

Selection on g is also negative in this case.

Selection on the trait v in the AC−C−U population can be determined even more easily. Since

$$\frac{\partial E(\mathrm{U}, \mathrm{AC})}{\partial v} = \frac{\partial E(\mathrm{U}, \mathrm{C})}{\partial v} = \frac{\partial E(\mathrm{U}, \mathrm{U})}{\partial v},$$

the gradient is independent of a, and consequently

$$i_v = 1/2.$$

Selection for saving energy by reducing competitive interactions is positive and independent of the composition of the population.

Discussion

In populations cultivated for commercial and many experimental purposes, there is usually an attempt to ensure that resources are sufficient for rapid growth and are equally available to all fish. This parallels ordinary animal husbandry practice which tends to reduce or eliminate the effect of social hierarchies and territoriality among domestic animals (Guhl 1969; Brown 1975). Under such conditions the fish which grow fastest will be those which are uninvolved in competitive social interactions and can minimize the costs of aggressive and submissive behaviour. Competition is not necessary to obtain sufficient food for rapid growth, or at least is substantially less necessary than in the natural environment of the fish. The resulting size variance is not caused by resource monopolization, but by "unnecessary" differences in utilization, metabolic patterns, and activity levels. Purdom (1979) recognized this and suggested that behavioural criteria might be more useful in selection programmes than growth itself.

We are indebted to the resource economist Chris MacCormac for pointing out to us that fish ponds are, ideally, managed to maximize the difference between the value of the gross yield (total physical product or TPP) and the cost of inputs including food. His experience in developing countries is that the input rate that maximizes this difference will almost always be less than the rate that maximizes the TPP (see also Smith 1982). There is a possibility, therefore, that aquaculture systems which are fed enough to select for Uninvolved fish may be uneconomic. If this happens routinely it could lead to a "tragedy of the commons" (Hardin 1968) in which individual short-term economic gain is obtained at the expense of long-term genetic quality of the stock. We would of course prefer that the critical TPP at which U fails as a pure strategy be lower than the TPP which is economically optimal.

The concern among aquaculture geneticists that increased competitiveness will cancel out attempts at selective stock improvement is probably unwarranted. We argue that much of the phenotypic variation in agonistic behaviour does not come about because some animals are genetically more aggressive than others, but because only the animals which happen to be larger, for genetic or other reasons, adopt an aggressive behaviour mode. Selection for increased growth rate among such phenotypes in moderately resource-rich environments will produce fish which are tamer, not more competitive. The essential point is that the Uninvolved strategy is stable and will be selected for, not the Contingent or pure Aggressor strategy, so long as the environment is managed noncompetitively.

Reduction in the intensity of Contingent behaviour might evolve fairly quickly in domestic environments. Malecha et al. (1979) found that both the skewness of the size distribution and the intensity of agonistic interaction were less in the domesticated strain of the Malasian prawn (*Macrobrachium rosenbergii*) than in the wild strains they tested. After 90 yr of cultivation, domesticated stocks of brook trout (*Salvelinus fontinalis*) are considerably faster growing, tamer, and less easily frightened than stocks of fish only one generation removed from the wild (Vincent 1960). Weatherly (1976) commented that it is probably no coincidence that the common carp (*Cyprinus carpio*), which has been under cultivation for more than 2000 yr, is extremely docile and tolerant of crowding.

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NOTES

An Empirical Model to Predict in situ Grazing Rates of *Diaptomus minutus* Lilljeborg on Small Algal Particles

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Chow-Fraser, P. 1986. An empirical model to predict in situ grazing rates of *Diaptomus minutus* Lilljeborg on small algal particles. Can. J. Fish. Aquat. Sci. 43: 1065–1070.

Grazing rates of *Diaptomus minutus* were measured in situ in six lakes in south-central Ontario using the tracer species *Chlorella vulgaris* and *Scenedesmus ovalis*. An empirical model was constructed to predict grazing rate as a function of the relative proportion of small (<10 μm) to large (10–30 μm) algal particles in the nanoplankton. It accurately predicted diaptomid grazing rates for three lakes in an independent lake set; however, it produced overestimates for two other lakes. There was also a significant positive correlation between grazing rate and the biomass concentration of small algae in the lakes. Difference in ambient lake temperature did not contribute significantly towards explained variation in grazing rate; prosome length was also a poor predictor of grazing rate. In parallel experiments, diaptomid grazing rates were higher when *Pediastrum* was used than when *Scenedesmus* was used as the tracer; in a similar set of experiments, grazing rates were higher when *Chlamydomonas* was used than when *Scenedesmus* was used.

Les taux de broutage de *Diaptomus minutus* ont été mesurés in situ dans six lacs du centre-sud ontarien à l'aide de deux espèces traceurs : *Chlorella vulgaris* et *Scenedesmus ovalis*. Un modèle empirique a été mis au point pour prédire les taux de broutage en fonction de la proportion relative de petites (<10 μm) et de grosses (10–30 μm) particules d'algues dans le nanoplancton. Le modèle a permis de prédire avec précision les taux de broutage des diaptomides dans trois lacs d'un groupe indépendant de lacs, mais a surestimé les taux dans deux autres cas. Nous avons également observé une corrélation positive significative entre les taux de broutage et la concentration de la biomasse de petites algues dans les lacs. Les différences des températures ambiantes des lacs n'ont pas contribué de façon significative à expliquer les variations des taux de broutage; de même, la longueur des prosomas était une variable explicative peu utile pour les taux de broutage. Des expériences parallèles ont montré que les taux de broutage des diaptomides étaient plus élevés lorsque *Pediastrum* était utilisé comme traceur plutôt que *Scenedesmus*. Une série d'expériences semblables a révélé que les taux de broutage étaient supérieurs lorsque *Chlamydomonas* était utilisé plutôt que *Scenedesmus*.

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Diaptomus is commonly found in the plankton of the northern United States and southern Canada. In many lakes of south-central Ontario, their biomass constitutes up to 50% of the total herbivore biomass during the summer (P. Chow-Fraser, unpubl.). Because of their numerical abundance, accurate measurement of their grazing rate is a prerequisite to realistic assessment of energy transfer between plankton compartments in these lakes.

Published data for freshwater calanoids are rare compared with those of marine calanoids and freshwater cladocerans (see Peters and Downing 1984). Additionally, grazing rates measured in the field (Richman 1964; Haney 1973; Thompson et al. 1982) are more rarely documented than those measured under laboratory conditions (Richman 1966; McQueen 1970; Richman et al. 1980; Vanderploeg 1981; Vanderploeg et al. 1984). Therefore, field measurements of diaptomid grazing rates are desirable so that extrapolation from laboratory data or from studies of other herbivorous taxa will not be necessary.

In laboratory studies, investigators found that clearance rates of *Diaptomus* vary according to temperature (Comita 1964), algal concentration (Richman 1966), and algal size (McQueen 1970; Richman et al. 1980). Few studies have attempted to determine whether or not these laboratory findings are applicable under natural situations. In this paper, I measure grazing rates of *Diaptomus minutus* and *D. oregonensis* in six lakes in south-central Ontario to examine the effects of temperature and algal composition on the in situ grazing rates of *Diaptomus*. An

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TABLE 1. Description of study lakes. pH measurements are summer averages of epilimnetic samples. Nanoplankton biovolume concentrations are in $mg \cdot m^{-3}$.

Lake	Mean depth (m)	Max. depth (m)	Volume (Mm^3)	pH	Nanoplankton biovolume <10 μm	Nanoplankton biovolume 10–30 μm	Blue-green filament biovolume
Model							
Head[a]	3.5	8.3	32.13	7.5	76.3	120.7	15.7
Moore[a]	7.5	21.6	13.80	6.0	42.3	104.6	0.4
Gull[a]	16.5	49.1	164.34	7.5	23.2	133.3	1.2
Blue Chalk[b]	9.3	21.9	4.70	6.0	47.5	319.0	0
Plastic[b]	8.1	16.7	2.64	5.6	13.3	124.9	0
Picard[b]	10.1	35.0	7.69	7.4	15.7	252.3	13.2
Test							
Hall's[a]	27.9	79.2	150.66	6.0	14.9	96.6	4.7
Mountain[b]	13.4	31.3	44.12	6.5	6.8	92.8	4.7
Brady[b]	4.5	11.5	4.08	5.8	32.5	146.1	1.4
Three-Mile[b]	3.4	10.9	32.33	6.6	56.5	25.7	211.9
Young[b]	4.3	10.4	4.00	8.1	559.0	820.0	287.0

[a]From Ontario Ministry of Natural Resources, Minden, Ont., unpubl. data.
[b]From Zimmerman et al. (1983) and Paloheimo and Zimmerman (1983).

empirical model was constructed to predict grazing rate of *D. minutus* from the relative abundance of small algal particles in the nanoplankton. I then test the applicability of this model by comparing predicted with measured rates obtained in an independently sampled lake set.

Methods and Materials

Six study lakes were sampled between late June and August in 1981 to provide data for the empirical model. Five additional lakes were sampled in 1982 to provide data for testing the applicability of the models. These lakes were all located in south-central Ontario and vary considerably in mean depth, volume, and pH values as well as algal standing stock (Table 1).

In situ grazing experiments were conducted with a 2-L Haney (1971) chamber at several depths in the water column of one station (usually at the deepest part of the lake). Experiments were usually performed between 10:00 and 16:00. The same procedures for grazing experiments, algal labelling, and determination of grazing rates were used in Chow-Fraser and Knoechel (1985). Ambient temperature was taken at the depth of the Haney chamber.

The labelled tracers used to measure grazing rates included *Chlorella vulgaris* (Carolina Biological; longest linear dimension 6–8 μm), *Scenedesmus ovalis* (Indiana Culture Collection; unicellular form, 4–6 μm), *Chlamydomonas* sp. (10–12 μm), *Pediastrum* sp. (coenobium, 30–40 μm; provided by P. Stokes, Botany Department, University of Toronto), and *Anabaena* sp. (vegetative cells approximately 4 μm in diameter, 6–8 μm in length, 25 cells·filament^{-1}; provided by S. Keal, Biology Department, Erindale College; toxic nature uncertain). The final concentration of *Scenedesmus*, *Chlamydomonas*, and *Chlorella* in the grazing chamber was calculated to be 1000 cells·mL^{-1}, 100 filaments·mL^{-1} for *Anabaena*, and 350 coenobia·mL^{-1} for *Pediastrum*. These represented an addition of less than 10% to the total phytoplankton biomass concentration in any experiment.

Parallel grazing experiments were conducted in Three-Mile and Mountain lakes with labelled *Chlorella* and *Scenedesmus* to determine the effect of using the two different tracers on grazing rates. No statistically significant differences were found between means (t-test, $P < 0.01$), and data from experiments with both tracer species were combined for construction of the model.

Zooplankton were stored on dry ice (Chow-Fraser and Knoechel 1985) and sorted without addition of preservative. I measured the prosome length of *Diaptomus* with the aid of a dissecting microscope. Grazing rates represent the average rates of groups of three to five animals of the same species of approximately the same size from a specific experiment. The developmental stage of the copepod was broadly noted as mature or immature but no attempt was made to determine the sex or the developmental stage of the immature instars.

Integrated phytoplankton samples from each lake were collected from the epilimnion (at the same place where grazing experiments were conducted). These were preserved and enumerated as in Chow-Fraser and Knoechel (1985). Besides total phytoplankton, the biomasses of algae with maximum linear dimension less than 10 μm (small nanoplankton; SNANO) and between 10 and 30 μm (large nanoplankton; LNANO) and blue-green filaments were also recorded (Table 1).

To test the effect of temperature on clearance rates (equivalent to grazing rates), experiments were conducted at 5, 10, 17, and 20°C in Picard Lake by lowering the grazing chamber to depths corresponding to these temperatures. Three experiments were conducted at each temperature. Only adult *D. minutus* were included in this analysis to avoid the confounding effects of developmental stage and species. I used a two-level hierarchical analysis of variance model to test for significant differences in grazing rates due to temperature effects and the nested effect of different grazing chambers within the four temperatures.

To test the effect of prosome length (equivalent to cephalothorax length) on clearance rate, I regressed data for both species of *Diaptomus* (conducted with labelled *Scenedesmus* and *Chlorella* only) against prosome length in a general linear regression analysis (Sokal and Rohlf 1981). Subsequently, I sorted the data and performed a separate regression analysis on *D. minutus* data only.

TABLE 2. Two-level nested analysis of variance to test the effect of temperature on clearance rate of *D. minutus* in Picard Lake.

Source	df	SS	MS	*F*	*P*
Among temperatures (5, 10, 17, 20°C)	3	0.1255	0.0418	0.07	0.98
Among grazing chambers within temperatures	8	9.7223	1.2153	1.89	0.11
Error	24	15.4051	0.6419		
% variance components					
Temperature	0				
Grazing chamber	23				
Unexplained variance	77				

I tested the effect of phytoplankton concentration on adult grazing rates of *D. minutus* in a correlation analysis. Clearance rates (measured with *Chlorella* and *Scenedesmus*) were correlated with total phytoplankton (TOTAL), nanoplankton biomass concentration (longest linear dimension <30 μm; NANO), SNANO (algae <10 μm), and LNANO (algae 10−30 μm) as well as the ratio of SNANO to LNANO (PROP).

The effect of tracer species on clearance rates of adult *Diaptomus* was tested by performing an analysis of variance on data obtained from grazing experiments in which labelled *Scenedesmus*, *Chlorella*, *Chlamydomonas*, and *Pediastrum* were fed to native zooplankton in Gull and Three-Mile lakes.

Results and Discussion

Temperature — The two-level nested analysis of variance indicated that temperature did not contribute significantly to the variation in grazing rate ($P = 0.98$; Table 2). Although differences among chambers were not statistically significant ($P = 0.11$), it accounted for 23% of the variation in the grazing rate of *D. minutus*. The overwhelming proportion of variation (77%) was unexplained by the model.

This analysis indicates that grazing rates measured at 5, 10, 17, and 20°C were statistically homogeneous. I suggest that any real effect of temperature was probably masked by individual variation among copepods as well as by microclimatic differences among chambers. Chow-Fraser and Knoechel (1985) also observed the lack of a significant temperature effect on the in situ grazing rates of cladocerans in lakes within this geographic region. Thus, the effect of temperature on diaptomid grazing rate is less predictable in nature than is demonstrated under controlled laboratory conditions (i.e. Comita 1964).

Prosome length — I regressed diaptomid clearance rates against prosome length ($n = 118$) and obtained the following:

$$(1)\quad \log CR = 2.07(\pm 0.90) \log L + 0.50(\pm 0.20)$$

where $r^2 = 0.04$, $P = 0.0001$, numbers in parentheses = SE, CR = clearance rate (millilitres per day), and L = prosome length (millimetres) (Fig. 1). Although the slope is significantly different from zero, prosome length only explained 4% of the variability in grazing rate.

Vanderploeg et al. (1984) also found that body size was a minor contributor to residual variation in the functional feeding curve of *D. sicilis*, mostly because of relatively invariant body size. L. B. Holtby (Pacific Biological Station, Nanaimo, B.C., unpubl. data) also conducted similar Haney grazing experiments with labelled yeast (maximum linear dimension approximately 3 μm), and when I regressed his data against measured prosome length, I found no statistically significant slope ($P = 0.57$). Body length generally appears to have weak predictive power for diaptomid grazing rate, and this contrasts sharply with the strong predictive power of length in describing cladoceran filtering rate (see Chow-Fraser and Knoechel 1985).

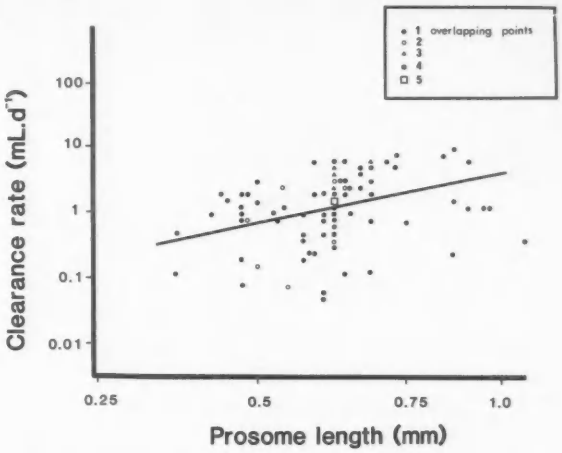

FIG. 1. Clearance rate of *Diaptomus* sp. versus prosome length. Solid line is the least-squares regression line through data; Eq. 1 in the text.

Regression of clearance rate against prosome length data for *D. minutus* ($n = 67$) increased the r^2 value relative to that for the combined mixed-species regression equation (Eq. 1) and resulted in the following:

$$(2)\quad \log CR = 3.00(\pm 1.26) \log L + 0.72(\pm 0.25)$$

where $r^2 = 0.09$ and $P = 0.02$. Since the r^2 value was significantly improved by 5% in the reduced dataset, a contributing factor to the large residual variation in Fig. 1 was probably inclusion of the two different species in the combined dataset. A probable reason is that sizes of developmental stages overlap considerably among species. Thus, the size of an early instar of *D. oregonensis* overlaps with that of a late *D. minutus* instar and yet may have comparatively different grazing rates. No attempt was made in this study to determine the relationship between grazing rate and developmental stages in *Diaptomus*.

Phytoplankton biomass concentrations — I next correlated grazing rate of *D. minutus* adults with various fractions of phytoplankton biomass concentration because food concentration in laboratory experiments was known to have an effect on clearance rates of *D. oregonensis* (Richman 1966). The best correlate of grazing rate was PROP ($r = 0.99$; Table 3), the next best being SNANO ($r = 0.80$). Regression of mean clearance rates for the six model lakes against PROP resulted in the following predictive regression (Fig. 2):

$$(3)\quad CR = 12.92\text{PROP} - 0.09.$$

I found supporting evidence for the positive correlation between grazing rate and relative proportion of small cells reported in other studies. Marshall and Orr (1955) found that *Calanus* supplied with a microflagellate (<10 μm) had greatest

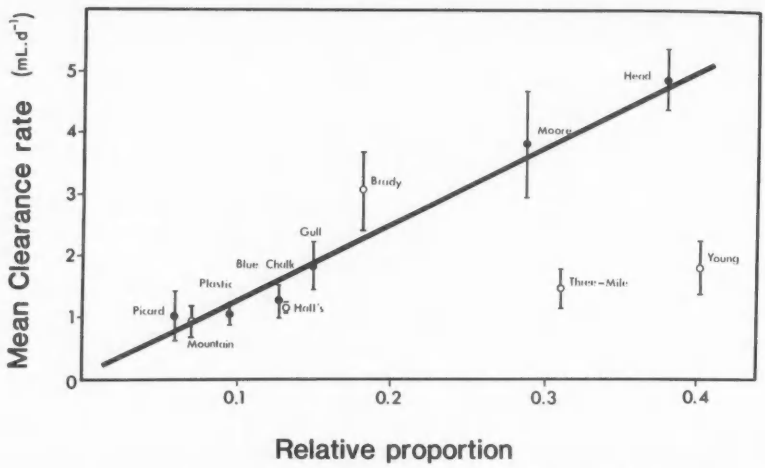

FIG. 2. Clearance rate of *D. minutus* versus the relative proportion of small cells (<10 μm) in the nanoplankton. Solid line is the least squares-regression line through solid circles only; Eq. 3 in the text. Open circles are values for the test lakes. Bars are 1 SE of the means. Labelled tracer species were *Chlorella* and *Scenedesmus*.

TABLE 3. Correlation between clearance rate of *D. minutus* and biovolume concentration of nanoplankton (<30 μm, NANO), small Nanoplankton (10 μm, SNANO), large nanoplankton (10−30 μm, LNANO), total phytoplankton (TOTAL), and the ratio of SNANO:NANO (PROP). *P* less than 0.05 indicates that the correlation coefficient, *r*, is significantly different from zero ($n = 6$).

Variable	*r*	*P*
NANO	−0.38	0.4598
SNANO	0.81	0.0529
LNANO	−0.60	0.2049
TOTAL	0.22	0.6766
PROP	0.99	0.0002

grazing rates when food was supplied at the highest concentration. In a time-series study, Richman et al. (1980) found that *D. oregonensis* increased its grazing rate on particles between 6 and 10 μm as the experiment progressed from 2 to 6 h. They noted that this increase in grazing rate of small cells was accompanied by a depletion of their supply of large cells (12−18 μm). Since the relative proportion of small cells increased as large cells became depleted, grazing rate on small cells in fact increased with the increase in relative proportion of small cells. Thus, both these studies provided evidence consistent with this study.

The positive correlation between grazing rate and SNANO biomass concentration (Table 3) is inconsistent with the conventional view that grazing rate is maximal and constant at phytoplankton concentrations below the incipient limiting concentration (ILC) and thereafter varies inversely with phytoplankton concentrations (Rigler 1961; McMahon and Rigler 1963; Richman 1966). It is, however, consistent with observations that grazing rates of certain marine calanoid copepods increase with phytoplankton concentration at extremely low algal concentrations (Parsons et al. 1967; Frost 1975; Reeve and Walter 1977; Gamble 1978). Since the phytoplankton concentrations in these lakes are as low or lower than those reported in these marine studies, it is probable that the increase in grazing rate with algal concentration noted here reflects a similar behavioral response in dilute environments as discussed by Frost (1975) for marine calanoids.

Test of the model — I plotted mean clearance rates from five test lakes (open circles) in Fig. 2 to test the applicability of the predictive model for other lakes in the region. Data for three, Hall's, Brady, and Mountain lakes, were close to the least-squares regression line (solid line) whereas those for Three-Mile and Young lakes were relatively distant. Therefore, the model appears to be applicable for three of the five test lakes.

Clearance rates for Three-Mile and Young lakes were much lower than predicted by the model. Both lakes had a high biomass concentration of small cells (Table 1) and a high relative proportion of SNANO compared with the other study lakes. Yet, the grazing rates were uniformly as low as those in lakes with much lower relative proportions of small cells. Both lakes were also unique in that blue-green filaments (such as *Anabaena, Oscillatoria*, and *Aphanizomenon*) were abundant.

I suspected that blue-green algal filaments in Three-Mile and Young lakes were responsible for reducing in situ grazing rate of *D. minutus* in a fashion similar to that observed by Richman and Dodson (1983). Tentative support for the negative effect of *Anabaena* on clearance rate is demonstrated by the statistically lower grazing rate when labelled *Anabaena* was used (mean 0.33 $mL \cdot d^{-1}$) in Three-Mile grazing experiments than when labelled *Scenedesmus* was used (mean 1.47 $mL \cdot d^{-1}$; *t*-test, $P = 0.03$). This represents a 4.5 times reduction in grazing rate between labelled tracers and indicates at the very least that labelled *Anabaena* was not ingested readily by *D. minutus* in Three-Mile Lake.

Although the empirical model should be modified to reflect other species of *Diaptomus* in many other geographical regions (something I am doing presently), the relatively close agreement of predicted to measured rates in at least some of the test lakes indicates that the model holds promise for predicting diaptomid grazing rates in lakes with a relatively low concentration of small planktonic algae.

Limitations of the model — The empirical relationship between clearance rate and relative proportion of small cells

TABLE 4. Mean clearance rates ($mL \cdot d^{-1} \pm 1$ SE) of *Diaptomus*.

Lake	Tracer species	Animal species	*n*	Clearance rate
Gull	*Scenedesmus*	*D. minutus*	10	1.76±0.38
	Pediastrum		10	4.81±0.78
	Scenedesmus	*D. oregonensis*	12	2.86±0.44
	Pediastrum		11	4.41±0.44
Three-Mile	*Scenedesmus*	*D. minutus*	9	1.47±0.31
	Chlorella		5	1.32±0.26
	Chlamydomonas		6	2.89±1.02

(Eq. 3) is only useful for predicting rates of *D. minutus* grazing on algae similar in size to *Scenedesmus* and *Chlorella*. When I measured grazing rates of *Diaptomus* using labelled *Pediastrum* and *Chlamydomonas*, both of which were larger than *Scenedesmus* and *Chlorella*, I obtained rates that were statistically different (*t*-tests and analysis of variance) than those measured in parallel experiments with labelled *Scenedesmus* or *Chlorella* (Table 4). Thus, differences in grazing rate due to size of algal tracer may limit the generality of the grazing model for predicting diaptomid feeding rates.

Discussion

Recently, Vanderploeg et al. (1984) used laboratory data to show that *D. sicilis* has an invariant selection for small algal particles. One probable explanation for this is that it feeds passively on small cells (4 μm) and that this feeding mode is constantly in operation (Vanderploeg and Paffenhöfer 1985). Invariant selection does not appear to apply to *D. minutus* in these lakes in view of Fig. 2. My results appear to be more consistent with findings of Richman et al. (1980) and Poulet (1973, 1974) that calanoid copepods feed opportunistically on the most abundant particles. However, since I did not conduct parallel experiments with large algal particles in all lakes, I do not know that *D. minutus* in fact changs its selection for small cells as a function of the relative abundance of small cells in the environment.

The apparent pattern of selection for small cells may be affected by the technique used to measure grazing rates. Vanderploeg et al. (1984) used an incubation period of longer than 18 h, whereas I used an incubation period of only 15 min in this study. Since selection for certain sizes of phytoplankton can change as the food size spectrum becomes altered when the preferred items are systematically depleted (Richman et al. 1977, 1980; Paffenhöfer 1984), selectivity observed at the end of an 18-h period may not necessarily reflect selectivity in the initial hours. Thus, grazing rates measured with the radiotracer method may more accurately reflect instantaneous selection, whereas those from coulter counter or direct microscope counting methods reflect the overall selection that has taken place during the long grazing period. Since the animal can respond to changes in the environment in minutes rather than hours (Cowles and Strickler 1983; Price et al. 1983; Vanderploeg and Paffenhöfer 1985; C. K. Wong, University of Toronto, unpubl. data), data that reflect instantaneous selection are probably more appropriate for interpreting the pattern of selection for animals grazing in a dynamic environment such as that in a lake.

High-speed filming similar to that used by Vanderploeg and Paffenhöfer (1985) should be conducted to determine whether or not *D. minutus* feeds on *Scenedesmus* and *Chlorella* actively and/or passively. Parallel experiments should also be performed to elucidate the relationship between relative abundance of seston fractions and the grazing rate of *D. minutus* on these size fractions to determine if "switching" or "peak tracking" occurs similar to that noted for other calanoids (Poulet 1973, 1974; Richman et al. 1977, 1980). More in situ experimentation with many diaptomid species will increase the rigor of the empirical relationship described here and hopefully lead to a more representative model of diaptomid grazing in lakes.

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Spatial Distribution of Entrained Fish Larvae in a Power Plant Discharge Canal[1]

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Larval fish were sampled with plankton nets during June and July at 1-, 3-, and 5-m depths at three stations in the 6-m-deep discharge canal of the Monroe electricity-generating plant on western Lake Erie. Of nine species, gizzard shad (*Dorosoma cepedianum*) accounted for 96% of all larval fish collected in June and, along with freshwater drum (*Aplodinotus grunniens*), 78% of those taken in July. Densities of fish larvae at the three depths, and at two of the three stations sampled, were not significantly different. Mean densities of gizzard shad and total fish larvae in June were significantly higher at one station. Fluctuating and significantly lower velocity at that station, causing the flowmeter not to turn while the net was still filtering water, was suspected of causing inflated densities. Generally sizes of larvae were not stratified by depth or station; differences that were found were small. Thus, we concluded that to obtain samples with representative species, densities, and sizes of entrained fish larvae, a stationary net should be positioned where the water has uniform high velocity and is well mixed.

On a prélevé des échantillons de larves de poisson au moyen de filets à plancton au cours de juin et juillet à des profondeurs de 1, 3 et 5 m à trois stations dans le canal de déversement de 6 m de profondeur de la centrale électrique Monroe située à l'ouest du lac Érié. Parmi neuf espèces, l'alose à gésier (*Dorosoma cepedianum*) constituait 96 % de toutes les larves de poisson recueillies en juin et, avec le malachigan (*Aplodinotus grunniens*), 78 % de celles capturées en juillet. La densité des larves de poisson aux trois profondeurs, et à deux des trois stations échantillonnées, ne différait pas de façon significative. La densité moyenne de l'alose à gésier et le nombre total de larves de poisson en juin étaient sensiblement plus élevés à une station. On a soupçonné la variation de la vitesse à cette station, de même qu'une vitesse beaucoup plus réduite, empêchant le débitmètre de tourner pendant que le filet filtrait encore l'eau, d'être les facteurs responsables des densités élevées. De façon générale, il n'y a pas eu stratification de la taille des larves en fonction de la profondeur ou de la station; les différences observées étaient peu importantes. Par conséquent, nous avons conclu que pour recueillir des échantillons où les espèces, la densité et la taille des larves de poisson transportées étaient représentatives, il fallait placer un filet fixe là où la vitesse de l'eau était uniformément élevée et où l'eau était bien mélangée.

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Documenting entrainment losses at electricity-generating plants is an important aspect of evaluating plant impact on fish. Studies are often conducted without sufficient knowledge of whether the sample adequately represents the species, densities, and sizes of entrained fish larvae. Sampler location should be based on criteria such as uniform current velocity and well-mixed water. Failure to do so could lead to a misleading estimation of the number of entrained fish larvae. During an impact study at the Monroe Power Plant located on the western shore of Lake Erie (Jude et al. 1983, available from the authors), we designed a special study to document the spatial distribution of larval fish in the discharge canal of the power plant. We avoided sampling

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in the intake canal because obtaining representative samples there is difficult. Vertical and horizontal stratification of fish larvae can occur in the intake water column due to entrance of water masses with different densities and species caused by upwelled water, seiches, and behavior of larval fish (e.g. pelagic versus demersal). To avoid these difficulties, we sampled in the discharge canal after intake water was presumably thoroughly mixed going through the plant condensers. Since many fish larvae are killed or debilitated passing through a power plant (Marcy 1973), stratification due to behavior should be reduced; however, fish larvae may be severely damaged, making identification impossible. Our objectives were to determine if fish larvae were homogeneously distributed in the discharge canal and to make recommendations where to take representative samples.

Methods

Larval fish sampling — Samples were collected on 7–8 June 1982 from 22:30 to 01:10 and 12–13 July 1982 from 22:15 to 00:05 in the 24-m-wide × 6-m-deep discharge canal. Based on early 1982 entrainment data (Jude et al. 1983), we sampled at night during June to maximize catch. The study was replicated in July to determine if larger larvae had a different distribution in the water column than newly hatched larvae in June. Water temperatures in the intake canal were 15.6 and 23.3°C, respectively, on the two dates. Throughout the study, all 12 circulating water pumps were continuously pumping at a total of 85 m^3/s. Discharge water leaves the Monroe plant via two concrete spillways which empty into a 305-m-long discharge canal which widens into a channel to Lake Erie. Three sampling stations were established at 6-m intervals along a 24-m-long bridge which spans the discharge canal just before it widens into the channel. At the sample location, the discharge canal bends, which causes the main flow of water to be deflected from the outer canal wall. Thus, water velocity was greater on the outside of the bend (station 3) than on the inside (station 1).

Sampling procedure was as follows. Three samples were collected simultaneously, one at each of the three stations. Samples were of 10-min duration in June, but had to be reduced to 5-min duration in July because of increased net clogging. Depths (1, 3, or 5 m) of the three simultaneously collected samples were assigned according to a Latin square design (Neter and Wasserman 1974). The sampling procedure was replicated four times, giving a total of 36 samples per sampling trip (three stations × three depths × four replicates). Three 363-μm-mesh, 0.5-m-diameter nylon plankton nets with attached Rigosha flowmeters were deployed using depressors to maintain position at the desired depths. We could not use a 1-m net because the weight necessary to submerge it under the high velocities in the canal was physically limiting. Volumes filtered per sample ranged from 2.4 to 80 m^3. Fish larvae were immediately preserved in a 10% formaldehyde solution. In the laboratory, fish larvae were removed, identified using Auer (1982), and measured to the nearest 0.1 mm total length. Fish were arbitrarily defined as larvae (<25.4 mm) or juveniles (>25.4–100 mm).

Water velocity (centimetres per second) was measured with a Price current meter on 16 July 1985 at the same locations and under similar flow regimes as existed during sample collection. Measurements were replicated three times.

Data analysis — The larval fish sampling design was developed for use in a multiple Latin squares analysis of variance (ANOVA) (Neter and Wasserman 1974). Data were tested for goodness-of-fit to the Poisson distribution (chi-square, Remington and Schork 1970); positive results would be an indication that fish larvae were well mixed throughout the water column. If results of this test were positive, a square root transformation of the data was used to meet the assumptions of ANOVA. All hypothesis testing was done using $\alpha = 0.05$.

Larval fish densities were tested for significant differences among stations, depths, order of net placement within a replicate (square), and replicates. The latter two effects are tests of temporal changes, i.e. large changes in densities of fish larvae over the course of the study period. Although a Latin square design assumes no interactions among effects within a square, a multiple Latin square design allows testing for all two-way interactions involving replicates. Testing for these interactions should ensure a representative mean square error so that confident hypothesis testing regarding depth and station can occur. If these interactions are not significant, then the significance of those assumed not to exist is unlikely (i.e. nonrandom shifting would have to occur on a time scale within replicates and be large enough to cover shifting on a time scale between replicates).

Larval fish density data were obtained for four independent, randomized Latin squares. If *F*-tests indicated a significant difference, Sheffe multiple comparison procedures were used to detect which levels of a factor were different. For June data, we tested gizzard shad (*Dorosoma cepedianum*), white bass (*Morone chrysops*) and white perch (*Morone americanus*) combined, yellow perch (*Perca flavescens*), emerald shiner (*Notropis atherinoides*), rainbow smelt (*Osmerus mordax*), common carp (*Cyprinus carpio*), and total fish larvae densities. For July data, we tested gizzard shad, white bass and white perch combined, freshwater drum (*Aplodinotus grunniens*), and total fish larvae densities. Similar tests were done for all species of juveniles combined. Because of low occurrence of fish larvae in July samples, data for larval rainbow smelt, emerald shiner, channel catfish (*Ictalurus punctatus*), and juvenile gizzard shad, and white bass and white perch combined violated ANOVA assumptions. This necessitated the use of the nonparametric Kruskal–Wallis test (Conover 1980) for main effects.

Mean lengths of fish larvae were also tested for significant differences among stations and depths to determine if any stratification occurred because of size-related larval fish behavior or sinking of dead larvae. Since sampling occurred over a short period (1.8–2.5 h), we assumed there would be minimal variability in mean length over time and therefore pooled these data over time. This resulted in a two-way, unbalanced ANOVA with the two factors being station and depth. For some species, a lack of data resulted in empty cells with this experimental design, thus dictating the use of a one-way ANOVA performed for both station with depths pooled and depth with stations pooled. From June, data for rainbow smelt, gizzard shad, yellow perch, white bass and white perch combined, emerald shiner, and common carp were tested, while for July, data for freshwater drum, gizzard shad, and white bass and white perch combined were tested. Velocity differences among depths and stations were tested with ANOVA at $\alpha = 0.05$.

Results

Densities — Gizzard shad made up 96% of the fish larvae collected in June, while gizzard shad and freshwater drum

TABLE 1. Mean densities (number/1000 m^3) and mean lengths (mm) of the most abundant species of fish larvae and juveniles collected from the discharge canal of the Monroe Power Plant in June and July 1982. Blanks indicate insufficient data for hypothesis testing. NA = not applicable; FO = frequency of occurrence; N = number measured. Total fish larvae and total juveniles include all species and damaged fish collected. *Significant difference across station, depth, or station × depth interaction. See Table 2 for densities or Table 4 for lengths.

Species	Mean density ± SD (FO)		Mean length ± SD (N)	
	June	July	June	July
Larvae				
Common carp	268±321 (25)		5.7±0.6 (62)	
Channel catfish	0	29±72 (6)		
Emerald shiner	159±297 (18)	43±92 (8)	7.2±2.2 (38)	
Freshwater drum	0	605±467 (32)		8.3*±2.0 (109)
Gizzard shad	59 200*±24 100 (36)	611±573 (30)	5.4*±1.6 (11 245)	12.5±4.2 (95)
Morone spp.	992±862 (33)	196±243 (22)	4.1±1.6 (234)	13.5±5.1 (32)
Rainbow smelt	460±619 (30)	21±64 (4)	14.2*±2.4 (82)	
Yellow perch	265±348 (26)		13.6±3.4 (67)	
Total fish larvae	61 500*±25 200 (36)	1570±986 (36)	NA	NA
Juveniles				
Gizzard shad	0	54±108 (8)		
Morone spp.	0	95±200 (11)		
Total juveniles	0	171±220 (22)	NA	NA

TABLE 2. Mean densities (number/1000 m^3) of gizzard shad larvae and total fish larvae collected by station from the discharge canal at the Monroe Power Plant in June 1982. Number of replicates for each station = 12. Values followed by the same letter are not significantly different ($P < 0.05$).

Species	Station	Density ± SD
Gizzard shad	1	86 600±21 800a
	2	44 700±10 400b
	3	46 300±5 760b
Total fish larvae	1	90 000±23 300a
	2	46 400±10 500b
	3	48 000±5 830b

TABLE 3. Mean water velocities (cm/s ± SD) at the stations and depths in the Monroe Power Plant discharge canal where larval fish samples were collected. Coefficient of variation (%) is given in parentheses.

Depth (m)	Station		
	1	2	3
1	40±4.2 (10.5)	51±7.3 (14.3)	90±6.0 (6.7)
3	31±4.8 (15.5)	52±3.4 (6.5)	76±3.4 (4.5)
5	47±9.8 (20.9)	81±1.1 (1.4)	96±3.3 (3.4)

constituted 78% in July (Table 1). No substantial differences in species composition among depths existed on either date, or among stations for June sampling. In July, rainbow smelt were not found at station 2, and channel catfish were not found at station 1.

Larval fish were homogeneously distributed throughout the water column, since density data for all species tested with multiple Latin squares ANOVA were found to be Poisson distributed. Latin squares hypothesis tests for interactions were nonsignificant for all species captured on both sampling dates, with the exception of rainbow smelt from June when the "replicate × order of net placement" factor attained significance ($P = 0.0449$) indicating differing temporal changes for replicates.

For data obtained in June, densities of all species and groups were similar among stations and depths with two exceptions. Gizzard shad and total fish larvae densities were significantly higher at station 1 ($P < 0.0001$) (Table 2). Water velocity at station 1 was significantly lower ($P = 0.0004$) and more variable (higher coefficients of variation) than at the other two stations (Table 3). A significant difference between replicates for gizzard shad ($P = 0.0454$) and total fish larvae ($P = 0.0499$) indicated that these densities changed over the sampling period.

Analysis of July density data showed no significant differences for any factor (ANOVA and Kruskal–Wallis tests, $P > 0.05$), although the station factor tests for gizzard shad and total fish larvae almost reached significance (0.0558 and 0.0514, respectively). Results for total juveniles indicated that temporal changes in densities occurred over the duration of sampling, as the replicate factor test was significant ($P = 0.0484$).

Sizes of larvae — Additional evidence for the homogenous distribution of fish larvae in the canal was the absence of significant differences in mean length of fish larvae with depth or station, with a few exceptions. Lengths of gizzard shad and rainbow smelt in June and freshwater drum in July showed some differences, most resulting from fish larvae collected at station 1 (Table 4).

Discussion

The two sampling dates provided representative fish species entrained, high larval fish densities, and both newly hatched and older larvae. Of 20 taxons (some fish larvae could not be identified to species) entrained in 1982 (Jude et al. 1983), 16 were collected in this study. Our June data are representative of peak densities of mostly newly hatched, planktonic fish larvae

TABLE 4. Mean lengths (mm) of three species of fish larvae collected by station and/or depth from the discharge canal of the Monroe Power Plant in June and July 1982. For each species, values followed by the same letter are not significantly different ($P < 0.05$). N = number measured.

Species	Station	Depth (m)	N	Mean length ± SD
		7–8 June		
Gizzard shad	1	1	626	5.4±2.2ab
	1	3	626	5.5±3.4a
	1	5	816	5.1±2.0b
	2	1	986	5.4±2.7a
	2	3	1935	5.5±2.4a
	2	5	1399	5.3±2.7ab
	3	1	1622	5.3±2.3ab
	3	3	2434	5.4±2.1a
	3	5	801	5.5±3.6a
Rainbow smelt	1		20	12.9±2.4a
	2		32	14.5±2.3b
	3		30	14.9±2.3b
		12 July		
Freshwater drum		1	35	9.1±2.3a
		3	31	7.9±1.8ab
		5	43	7.9±1.6b

passing through the plant, a conclusion we base on concurrently collected entrainment data (Jude et al. 1983). About 90% of all fish larvae were entrained from 17 May to 30 June (Jude et al. 1983). The July data are indicative of patterns of distribution after most fish larvae have grown out of the planktonic stage; July was also the month of third-highest entrainment losses (Jude et al. 1983).

On both sampling dates, larval fish were homogeneously mixed throughout the vertical water column at all stations, and lengths of most species did not vary significantly among stations or depths. The significant difference in length for gizzard shad at one location was only 0.3–0.4 mm. The mean length differences for rainbow smelt and freshwater drum were 1–2 mm and may have reflected the ability of slightly larger larvae to select a depth stratum or current velocity.

Station 1 had higher larval fish densities on both sampling dates, although only gizzard shad and total fish larvae for June were significantly higher. Station 1 was on the inner bend of the discharge canal, where mean water velocity (39 cm/s) was the slowest and the most variable compared with the other two stations (61 and 87 cm/s). Fluctuating velocity at station 1 was also apparent from visual observations. The low and variable velocity may have caused higher densities because (1) fish larvae gathered in slow current or (2) velocity was sometimes insufficient to keep the flowmeter turning while the net was still filtering larvae, and an artificially low meter reading would result in higher apparent densities. We believe that the latter reason is the cause, because entrained fish larvae are either dead or stressed and would be unlikely to actively gather in slow current. Some volumes filtered during 10-min samples at station 1 were less than 3 m^3, a level that, we know from experience, causes intermittent (rather than constant) turning of the meter propeller.

On 7 June, the mean density of total fish larvae over all depths at station 1 was 90 000/1000 m^3, compared with 46 400/1000 m^3 at station 2 and 48 000/1000 m^3 at station 3. The grand mean for all stations (an estimate for the entire canal) was 61 500/1000 m^3, 33% higher than the station 2 density. However, mean density of fish larvae in all 12 July samples (1570/1000 m^3) was only 5% higher than the station 2 density (1490/1000 m^3). Results from these two sampling dates demonstrate that the density difference among stations was not consistent, and significant differences were possibly dependent on the total number of larvae passing through the discharge canal. Because of this difference between sampling dates, and the uncertainty about meter readings at station 1, entrainment estimates derived from stations 2 or 3, where velocities were uniformly high, would give the most accurate estimate of larval fish losses.

During the design of the study, we eliminated from consideration sampling in the intake canal, which draws water mainly from the Raisin River but also from Lake Erie during low river discharge. Different water masses have differing physical characteristics and larval fish species complexes. These water masses enter the intake canal and cause stratification of fish larvae vertically and horizontally. Thus, we decided to sample the water after it became mixed passing through the plant's pumps and condensers. This mixing should randomly distribute fish larvae throughout the water column and thus reduce sampling variability. A potential drawback of sampling after plant passage is that larvae may be damaged, preventing accurate identification. We found no such problem, as 0.1 and 1.5% of the respective June and July grand mean densities were fish larvae damaged beyond recognition. In comparable entrainment studies at the Campbell Plant, Lake Michigan, damaged larvae made up 0.9–5.5% of the total entrainment loss for 1977–81 (Jude et al. 1980, 1981, 1982).

Although this experimental design for data analysis is somewhat restrictive because it assumes no within-replicate interactions, it does address such problems as sampling difficulty, it controls variation due to temporal differences, and we were able to test for interactions that would indicate a possible violation of the assumptions (shifting of density among stations over time). Had substantial shifting of larval fish densities among stations over time been detected, perhaps a lattice sampling design (Yates 1965) would have been more appropriate.

Leslie (1983) showed that there is considerable variability in current velocity depending on forebay configuration, volume of water discharged, and position in the water column. Despite some horizontal variability in current velocity in the Monroe Power Plant discharge canal, we found no significant differences in densities of larval fish with depth, but there were some differences in density among stations, which were mainly due to low and fluctuating velocities in one area, station 1. Velocities at the other two stations were similar and varied much less. We thus recommend that areas of fluctuating and low velocity in discharge canals be avoided, particularly during times of peak densities. If velocity is uniform, high enough to turn the flowmeter consistently, and the water well mixed, sampling position could be anywhere in the vertical water column, but deployment of the heavy depressor–net apparatus would be easiest at the surface. Sampling should be done at night to maximize catch.

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Spatial Correlations of Transferrin Allele Frequencies in Atlantic Salmon (*Salmo salar*) Populations from North America

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Verspoor, E. 1986. Spatial correlations of transferrin allele frequencies in Atlantic salmon (*Salmo salar*) populations from North America. Can. J. Fish. Aquat. Sci. 43: 1074–1078.

Existing analyses of spatial patterns of transferrin (Tf) allele frequency variation in anadromous Atlantic salmon (*Salmo salar*) from rivers in North America have not utilized all available data. Further analysis shows that a latitudinal cline in Tf-4 allele frequency previously reported is an artefact of a major regional difference between populations from Newfoundland/Labrador and those sampled elsewhere. Furthermore, the allele frequencies of populations outside Newfoundland/Labrador show a significant association with the proportion of spawning fish in the populations having spent only one winter at sea. Fequencies in Newfoundland/Labrador are collectively consistent with this relationship but by themselves show no similar correlation. These results, combined with previously reported differences between anadromous and nonanadromous fish, suggest that selective forces, which vary with life history pattern, may operate on the polymorphism.

Les analyses existantes de la variation spatiale de la fréquence des allèles de la transferrine (Tf) chez le saumon de l'Atlantique (*Salmo salar*) anadrome dans des cours d'eau d'Amérique du Nord n'ont pas porté sur l'ensemble complet des données disponibles. Une analyse plus poussée montre qu'une cline latitudinale dans la fréquence des allèles de la Tf-4 signalée antérieurement est un artefact lié à une importante différence régionale entre les populations de Terre-Neuve et du Labrador et celles échantillonnées ailleurs. En outre, la fréquence des allèles chez les populations à l'extérieur de Terre-Neuve et du Labrador présente une association significative avec la proportion de poissons en frai dans la population, qui n'ont passé qu'un hiver en mer. Les fréquences observées à Terre-Neuve et au Labrador sont collectivement en accord avec cette relation, mais ne présentent en soi aucune corrélation semblable. Ces résultats, combinés aux différences observées auparavant entre les poissons anadromes et les poissons non anadromes, indiquent que des forces sélectives, qui varient en fonction de l'histoire naturelle des espèces, peuvent agir sur le polymorphisme.

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The most extensively studied genetic polymorphism in the Atlantic salmon (*Salmo salar*) is that of transferrin (Tf), the protein transporting iron in the blood. Yet, even for this locus the factors which determine allele and genotype frequencies within populations are not understood. This is at least partly attributable to incomplete assessment of the results of existing work. Presented here is a new analysis of existing data on transferrin allele frequency variation among populations of *S. salar* in North American rivers. This represents the first time an analysis of all the available data has been made and provides new insight into interpopulation variation at this locus.

The transferrin polymorphism in *S. salar* has been studied in the British Isles (Payne et al. 1971; Child et al. 1976) as well as in North America (Møller 1970, 1971; Payne 1974). Møller (1970) showed that North American populations have three common phenotypes which arise from the segregation of two co-dominant alleles, most recently designated Tf-1 and Tf-4. A third rare allele, Tf-3, also occurs and gives rise to some uncommon phenotypes. In Europe, only the Tf-1 and a further allele, Tf-2, occur. The distribution of genetic variation in Britain shows a regional pattern (Child et al. 1976), and Møller (1970) suggested that this was also the case in North America. Payne (1974) later rejected this view and argued that there was a latitudinal cline in North America, but he did not incorporate all the population data available in his analysis.

Methods

The data are from samples of anadromous fish taken from North American rivers and reported by Møller (1970, 1971) and Payne (1974). The data were restricted to samples of 30 or more fish, to one river per river system to make samples maximally independent, and to data on juvenile fish where possible, as this was the most frequently sampled life history stage. In the case of the St. John River, several samples of adults were taken in each of two years and the samples were homogeneous within years but heterogeneous between years. A mean frequency for the two years is therefore used for the frequency estimate for the river. Values for the proportion of one-sea-winter fish (grilse) in the spawning populations of the rivers used in the analysis (Table 1) are derived from angling data for the years 1968–74 inclusive. Statistical analyses have employed a $\sin^{-1}\sqrt{\ }$ transformation of frequency data to normalize their distributions (Sokal and Rohlf 1981).

Results and Discussion

The available data on Tf-4 allele frequencies include populations from most of the southern and central part of the range of *S. salar* in North America, but no data exist for northern populations from Ungava Bay in northern Quebec, the north shore of the Gulf of St. Lawrence, and most of Labrador. The transferrin allele frequency variation in the area surveyed is regionally distributed (Fig. 1), with rivers in Newfoundland and Labrador having much lower Tf-4 frequencies than elsewhere. This difference is seen most clearly on the insert graph on Fig. 1 where the frequencies of four arbitrary regional groupings are compared. While none of the other three geographical areas are detectably different, all are different from Newfoundland/Labrador, although some overlap occurs. The difference in mean frequency between Newfoundland/Labrador (0.157) and the populations elsewhere (0.448) is highly significant ($t = 6.56$, $n = 31$, $P < 0.001$).

The existence of an overall latitudinal cline in Tf-4 frequency, first argued by Payne (1974), is not supported by analysis of the more extensive data set used here. An association appears to exist ($r = -0.534$, $n = 31$, $P < 0.01$) but is largely an artefact of the allele frequency differences between rivers in Newfoundland/Labrador and those elsewhere. If an overall cline were present, it should be detectable within each of these geographical groups as well as in the combined data. This is not the case. The Newfoundland/Labrador rivers show a moderate correlation ($r = -0.583$, $n = 12$, $P < 0.05$), but in the larger sample of rivers elsewhere no significant correlation at all is found ($r = 0.234$, $n = 19$, $P > 0.50$). The overall association in the data is, therefore, largely a product of the mean difference between the two regional groups and their north–south displacement. The existence of a regional cline within Newfoundland/Labrador is, however, clearly possible.

The data justify the division of the North American populations into the two regional groups on the basis of Tf-4 allele frequencies. The difference between them could have arisen through historical patterns of colonization and gene flow as has been suggested for regional differentiation in the British Isles (Payne et al. 1971; Child et al. 1976), or it could be the result of current evolutionary forces as Møller (1971) put forward. Association of allele frequencies with environmental variables, life history stages, or life history patterns would provide support for the latter position (Clarke 1975).

Power (1981) tested for an association of Tf-4 allele frequencies with each of 36 environmental variables relating to climate and physical river characteristics, but found none. The possibility of an association of allele frequency with a life history trait — the proportion of the spawning population composed of fish returning to spawn for the first time after one-winter-at-sea (grilse) — is tested here. Populations in Newfoundland and Labrador differ from populations elsewhere by having a high proportion of grilse. This suggests a possible association of increasing Tf-4 frequency with decreasing proportions of grilse in the spawners; a plot of the two variables, for the 28 sites for which percent grilse data could be obtained (Fig. 2), shows that an overall relationship does exist. In the *non*-Newfoundland/Labrador sites, a similar relationship is present in the three regional subgroups previously defined (Fig. 1), and the combined correlation for these three groups is significant ($r = -0.578$, $n = 16$, $P < 0.02$). The Newfoundland/Labrador rivers collectively are consistent with this relationship (Fig. 2) but in themselves show a moderate, although nonsignificant, positive correlation between the two variables ($r = 0.417$, $n = 12$, $P < 0.20$).

These results require a reevaluation of the cause(s) of inter-river variation in transferrin allele frequencies in North American Atlantic salmon. Firstly, as Møller (1970) originally suggested, a definite regional differentiation exists between Newfoundland/Labrador salmon and those examined elsewhere in North America. Secondly, no broad latitudinal cline exists in North American populations, as argued by Payne (1974). Finally, inter-river variation outside Newfoundland/Labrador may in itself not be randomly distributed but associated with population variation in the proportion of one-sea-winter fish in the spawning population.

The existence of regional differences in North America parallels the situation found in the British Isles where salmon in rivers from southern Ireland, Wales, and southeast England differ from those elsewhere (Child et al. 1976). This regional difference also gives rise to an artifactual latitudinal cline in the

TABLE 1. Summary of data on Tf-4 allele frequencies, percentage grilse in spawning population, and latitude for rivers in eastern North America for which data on the transferrin polymorphism in *S. salar* exists.

River	Approximate latitude	Tf-4 frequency[a]	No. of fish in sample	% grilse (ref.[b])
Newfoundland/Labrador				
Sand Hill River	53°35′	0.085	130	94 (2)
St. Genevieve River	51°11′	0.170	81	95 (2)
West River	51°11′	0.070	156	98 (2)
Indian River	49°30′	0.142	120	100 (2)
Aides Stream	49°20′	0.071	112	92 (2)
Salmon Brook	49°17′	0.070	67	98 (2)
Dog Bay River	49°17′	0.230	91	100 (2)
Exploits River	49°08′	0.130	100	94 (2)
Middle Brook	48°46′	0.090	95	99 (2)
Terra Nova River	48°40′	0.240	54	98 (2)
Come by Chance River	47°54′	0.360	196	100 (2)
White Bear River	47°35′	0.370	95	97 (2)
Quebec				
MacDonald River	49°45′	0.650	143	48 (4)
Jupiter River	49°25′	0.587	154	29 (4)
Dartmouth River	48°50′	0.503	164	20 (4)
Matane River	48°50′	0.332	122	28 (4)
St. Jean River	48°45′	0.587	115	16 (4)
Grand Cascapedia River	48°15′	0.423	146	6 (4)
New Brunswick				
Restigouche River	48°02′	0.408	120	25 (3)
Sevogle River	47°00′	0.344	44	88 (3)
Bartholomew River	46°30′	0.355	90	57 (3)
Big Salmon River	45°30′	0.300	120	44 (3)
Dennis Stream	45°15′	0.500	40	NA —
St. John River	46°16′	0.330	458	56 (3)
Nova Scotia				
Margaree River	46°26′	0.452	115	35 (3)
Middle River	46°10′	0.536	110	4 (3)
Philip River	45°45′	0.325	120	56 (3)
Wallace River	45°40′	0.297	70	NA —
East River	45°20′	0.292	120	NA —
Maine				
Dennys River	44°54′	0.645	76	2 (5)
Machias River	44°40′	0.645	124	3 (1)

[a]Data from Møller (1970, 1971) and Payne (1974).

[b]Data source: 1, Schaffer and Elson (1975); 2, Moores and Ash (1984); 3, Smith (1981); 4, Y. Côté (Gouvernement du Québec, Ministère du Loisir, de la Chasse et de la Pêche, Québec, Qué., pers. comm.); 5, K. F. Beland (Atlantic Sea Run Salmon Commission, P.O. Box 1298, Bangor, ME 04401, pers. comm.); NA, no data available.

British Isles as a whole. In the British Isles, the pattern has been argued to have arisen from historical colonization patterns from two glacial refugia in combination with subsequent restricted gene flow between populations in different rivers. A similar argument could possibly be made for North American regional differences. However, the association detected here between allelic variation and variation in a life history trait points to more recent, regionally varying selective forces being involved.

The case for selection influencing Tf-4 frequencies of populations is, as yet, circumstantial. The absence of a correlation of Tf-4 frequency with percent grilse within the Newfoundland data set, even though the data collectively are consistent with the relationship shown by the rivers elsewhere, is apparently contradictory. This result may have arisen because of the narrow range of variation in the proportion of grilse in Newfoundland/Labrador rivers which works against detecting an association. However, it is also possible that the frequencies in Newfoundland are subject to influence by factors not generally experienced by populations in rivers elsewhere. One such factor may be interbreeding with nonanadromous fish. Nonanadromous fish are present in many of the river systems in Newfoundland and Labrador but mostly absent elsewhere. Interbreeding between the two life history types can occur (Hutchings and Myers 1985), and nonanadromous stocks show very high Tf-4 frequencies (0.78–0.91, Payne 1974). Tf-4 frequencies in anadromous salmon from rivers which also contain the nonanadromous form (e.g. Exploits, Terra Nova, and White Bear rivers, Payne 1974; T. R. Porter, Department of Fisheries and Oceans, P.O. Box 5667, St. John's, Nfld., pers. comm.) are higher than those where the nonanadromous form appears to be absent (Sand Hill River, Aides Stream,

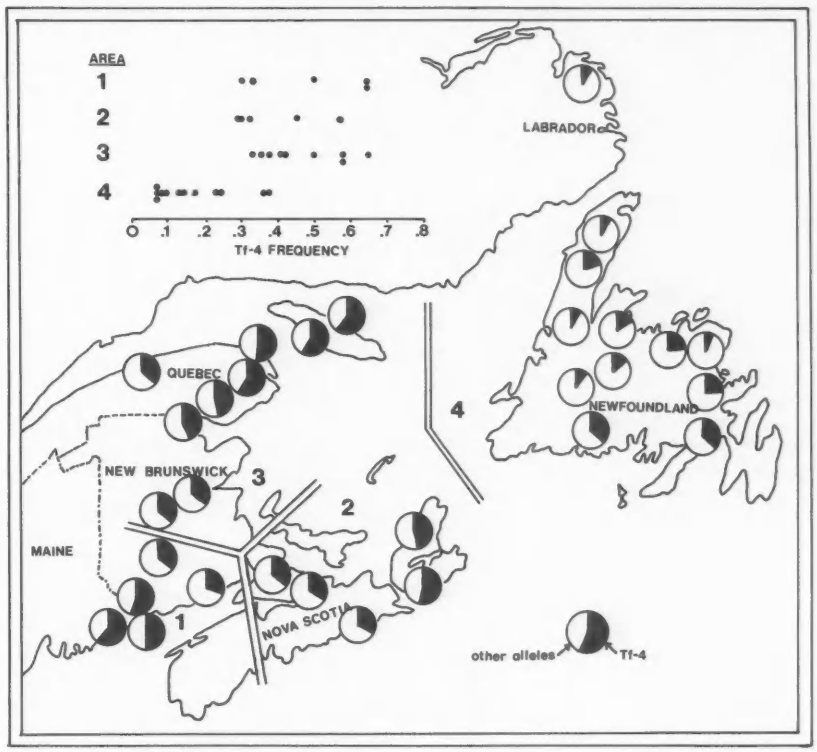

FIG. 1. Distribution of Tf-4 allele frequencies among populations of *S. salar* in eastern North America.

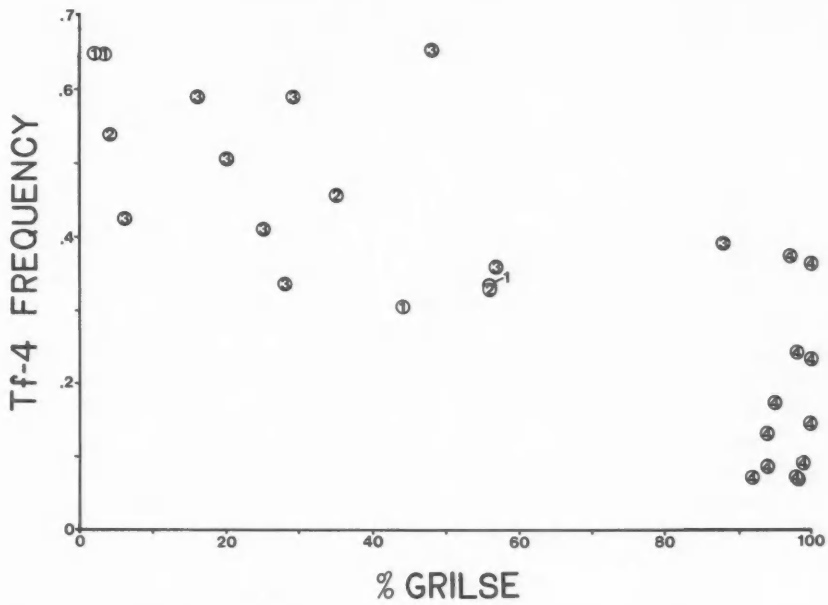

FIG. 2. Relationship between Tf-4 allele frequency and the percent grilse in populations of *S. salar* in eastern North America. Numbers refer to regions shown in Fig. 1.

West River, and Salmon Brook, T. R. Porter, pers. comm.). This suggests that interbreeding may be responsible for the elevated Tf-4 frequencies in some anadromous populations in Newfoundland. At the same time, the existence of this major genetic difference between anadromous and nonanadromous forms lends further support to an association of variation at the Tf locus with life history variation.

What the selective forces associated with life history might be that act on variation at the transferrin locus, and whether they act directly on the locus or not, can only be speculated on at this time. Transferrin genotypes in other salmonids have shown differential resistance to infections such as bacterial kidney disease (Suzumato et al. 1977; Winter et al. 1980), a disease present in many populations of Atlantic salmon in Nova Scotia and New Brunswick (J. W. Cornick, Department of Fisheries and Oceans, Halifax, N.S. B3J 2S7, unpubl. data; Paterson et al. 1981) as well as in the British Isles (Smith 1964). It is known that progression of BKD is rapidly reversed following acclimation of infected salmon to seawater although mortality of infected individuals during acclimation is high; also, returning spawners previously infected incur a higher river mortality than nonaffected fish (Frantsi et al. 1975, 1977). Whether the amount of time spent at sea prior to spawning affects BKD-induced mortality rates is not known. The potential advantage of superior transferrin binding of blood iron in resistance to bacterial infection has been recognized for some time (Fletcher and Huehns 1968; Weinberg 1974), and heterozygote superiority in iron binding capacity of transferrin genotypes has been reported in the brook trout, *Salvelinus fontinalis* (Hershberger 1970). Collectively, these observations suggest that a functional connection between life history pattern, selective mortality in relation to disease, and transferrin allele frequencies in *S. salar* populations could exist. However, they are insufficient for identifying a likely selective pathway which could produce the observed association. The possibility that selection is operating should be further explored as, if true, it would be important to the use of the polymorphism in stock discrimination work and in the development of stocks for culture programs. Also, it would suggest that factors which lead to the alteration of the proportions of life history types in a population, such as heavy or selective fishing, could alter the genetic nature of Atlantic salmon populations.

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Changes in Lengths of Fixed and Preserved Young Freshwater Fish

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The lengths of body parts of several species of young freshwater fish were measured to determine short- and medium-term effects of fixation and preservation. Shrinkage (up to 4.9%) was the rule, but lengths also increased, or remained static; rigor mortis was probably the main cause of immediate change. However, measurement error probably affected precision in the smallest lengths. Body parts changed differentially; these changes were not species specific, nor was type of fixative or preservative significant. Trunk and snout to vent length indicated most susceptibility to change (especially shrinkage); head length was least affected. Changes were considered of little or no practical consequence for taxonomy and growth studies.

On a mesuré la longueur de certaines parties du corps chez plusieurs espèces de jeunes poissons d'eau douce pour déterminer les effets à court et à moyen terme de la fixation et de la conservation. En règle générale, il y a eu rétrécissement (jusqu'à 4,9 %) mais on a observé également des cas où la longueur a augmenté ou n'a pas changé; la rigidité cadavérique était probablement la principale cause du changement immédiat observé. Cependant, des erreurs de mesure ont probablement influé sur la précision dans le cas des longueurs les plus faibles. Les changements ont différé selon la partie du corps; ces changements n'étaient pas liés à l'espèce et le type de fixateur ou d'agent de conservation n'était pas non plus un facteur important. La longueur du tronc et celle du museau à l'anus étaient les mesures les plus susceptibles de changer (surtout en ce qui concerne le rétrécissement); la longueur de la tête est celle qui a le moins varié. On a considéré que ces changements avaient dans la pratique peu ou pas de conséquences pour ce qui est de la taxonomie et des études sur la croissance.

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Fixation and preservation effects on marine larval fish have been reported in a number of recent studies (Radtke and Waiwood 1980; Theilacker 1980; Hay 1982; Fowler and Smith 1983), and mostly concerned shrinkage in body lengths. It is not known if freshwater larval fish respond to fixation and preservation in the same way as their marine counterparts.

We provide a practical evaluation of the effects of several fixatives and preservatives on the lengths of various body characters in a number of freshwater larval fish species. The study was intended primarily to document artificial morphological changes, which may interfere with identification, accurate taxonomic description, and correct diagnoses of natural deformities in young fish (Barahona-Fernandes 1982). The role of measurement precision in relation to changes in fixed and preserved specimens is also considered.

Materials and Methods

Short-term changes — Several species of larval fish (Table 1) were seined from creeks near Hamilton, Ont., and taken alive to the laboratory within 2 h of capture. Fish were anaesthetized with 50% carbonated water or MS-222, and various body components measured; individual fish were then placed in numbered vials with specific fixatives (Table 1) and remeasured after 1 or 2 d and at least three more times within 45–60 d. *Pimephales notatus* were equally divided into three groups, measured live, and then placed in three separate fixatives: 4% formalin buffered with marble chips, 60% ethanol, and Davidson's B (Henderson 1963). These fixatives were chosen to compare their ability to conserve the morphological integrity of young fish during the short-term period, i.e. the first 6–8 wk postmortem.

Medium-term changes — To observe continuing length changes in addition to those incurred at the time of fixation, 145 *Catostomus commersoni* larvae (14.1–20.2 mm TL) were hand-dipped from a local stream, divided into four groups of 35–40 fish each, and fixed on site, using solutions of Davidson's B, 60% ethanol, 4% formalin, and 10% Borealene (Boreal Laboratories, Mississauga, Ont.). Subsequent measurements, using a dissecting microscope equipped with an ocular micrometer, were taken 10 wk postfixation and then 2, 4, 6, 10, 13, 17, 40, and 61 wk later. This procedure covered a time span typical of routine laboratory processing of field samples. Similarly, 22 juvenile *Esox americanus vermiculatus* from Twenty Mile Creek (near Hamilton, Ont.) were fixed and preserved in 10% Borealene and subsequently measured 6, 8, 10, 12, 16, 23, 28, 66, 75, and 101 wk later.

The following linear measurements were made: *total length (TL)*: distance between tip of snout and most posterior tip of caudal finfold or fin; *standard length*: tip of snout to posterior extremity of notochord or hypurals; *head*: tip of snout to base of pectoral fin or centre of cleithrum, as each structure developed; *orbit*: greatest horizontal distance between orbital rims; *snout to vent*: tip of snout to centre of anus; *least body depth*: minimum depth at caudal peduncle; *greatest body depth*: maximum depth, excluding yolk sac and finfolds; *yolk sac*: anterior to posterior extremity of yolk sac.

To locate specific areas of change, measurements were also

TABLE 1. Short-term shrinkage in length of preserved freshwater fish. Mean live length (in parentheses) in millimetres; n = number of fish; NC = no change.

Species	n	Mean change in length (%)						Preservation type and duration (d)
		Total	Standard	Snout to vent	Head	Greatest depth	Yolk sac	
Composite sample	21	NC (5.5)	NC (5.3)	NC (3.9)				Davidson's B (45)
Etheostoma nigrum	20	NC (6.1)	NC (5.9)	NC (3.1)	NC (1.1)		NC (1.7)	Davidson's B (45)
Rhinichthys atratulus	17	2.6 (20.9)	3.1 (17.5)	NC (11.4)	NC (4.5)	NC (3.6)		Davidson's B (45)
Catostomus commersoni	8	1.8 (27.2)	3.2 (22.7)	3.4 (17.4)	NC (6.3)	3.4 (4.4)		Davidson's B (45)
Esox americanus	15	1.5 (10.6)	NC (10.3)	2.5 (6.8)	NC (2.7)			4% formalin (60)
Esox lucius	15	3.5 (15.2)	3.5 (14.9)	3.8 (10.3)	NC (3.6)			4% formalin (60)
Pimephales notatus	30		4.3 (26.7)					Davidson's B (53)
	30		4.1 (26.5)					4% formalin (53)
	30		4.9 (26.5)					60% ethanol (53)

taken of the following lengths: *body*: distance from origin of pectoral fin to posterior margin of caudal fin; *trunk*: distance between centre of cleithrum and centre of anus; *postanal*: distance from centre of anus to tip of caudal fin.

Fixation refers to prevention of change in tissue at, and immediately subsequent to, death, whereas preservation is the process of maintaining the integrity of the fixed specimens for long-term storage. All fish were preserved in their own fixative and stored in the dark at 25°C. Live lengths of each body part of individual fish were compared with the mean lengths of preserved fish, each of which was held in a numbered vial. Measurements greater than 10 mm were made to 0.1 mm; thus, comparative lengths indicating a maximum difference of ±0.14 mm were considered unchanged. All remaining lengths were made to 0.01 mm. A one-sided paired t-test was applied, where applicable, to the data. For each measured variable during the medium-term period, a two-way analysis of variance was used to detect significant length changes.

Results

Short-term changes — There were no measurable changes in mean lengths of body parts of a composite larval fish sample (5.5 mm mean TL) in Davidson's B solution over 45 d (Table 1). Specimens of *Etheostoma nigrum* (6.1 mm mean TL) retained their live total, standard, snout to vent, head, and yolk sac length during preservation in Davidson's B for 45 d. Individual fish shrank as much as 6%, and increased up to 3% in total length throughout the measurement sequence; most change occurred within 2 d of fixation.

Measurements of *Rhinichthys atratulus* and *C. commersoni* indicated a maximum mean shrinkage of 3% over 45 d (Table 1). In *R. atratulus*, trunk and postanal areas of the body shrank 0.3 mm (4.4%) and 0.2 mm (2.1%), respectively. Similarly, *C. commersoni* shrank 5.3% in trunk length, but increased 1% postanally during 45 d. In all cases, more than two-thirds of the change occurred within 2 d of fixation.

Esox a. vermiculatus (10.6 mm mean TL) shrank 2.5% in snout to vent length (Table 1), virtually all of which occurred in the first day. The head increased 3.8% in the first 24 h and then decreased slightly during the next 30 d, after which no change occurred. Further, the body shrank (2.7%), as did trunk length (5.3%), whereas postanal length did not change; shrinkages in total and snout to vent length were highly significant (t-test; $P < 0.01$).

Esox lucius (15.2 mm mean TL) shrank 3–4% in total, standard, and snout to vent length, but head length did not change (Table 1). Live (anaesthetized) lengths and those 60 d postfixation were significantly different (t-test; $P < 0.01$), but there was virtually no change after the first day of fixation. Mean shrinkage in trunk and postanal length during 60 d in 4% formalin was 2.9 and 5.8%, respectively, and this occurred during the first day (22–24 h) postmortem. External morphology of all specimens appeared unaffected by preservatives.

Shrinkages in standard length of 90 *P. notatus* (26 mm mean TL) larvae during 53 d were similar (4–5%), whether fixed and preserved with Davidson's B, 4% formalin, or 60% ethanol. These changes were significant (t-test; $P < 0.01$). There were no length changes following the first postfixative measurement (day 4).

Medium-term changes — During 61 wk, changes in mean total and snout to vent lengths in 145 preserved larval *C. commersoni* were either unchanged or negligible in each of Davidson's B, 4% formalin, and 10% Borealene, and barely perceptible (0.2-mm shrinkage in total length) in 60% ethanol. Sequential measurements of lengths indicated no change or slight fluctuation about initial values, which were taken 10 wk after fixation. No temporal effects of preservation were indicated (ANOVA; $P < 0.01$).

The result of medium-term (101 wk) preservation of 22 juvenile *E. a. vermiculatus* (52.0 ± 2.7 mm TL) was shrinkage from initial head length (2.6%), orbit, and least depth (5–6%), and increase in total length (1%) and body (2.3%), all of which were highly significant (ANOVA; $P < 0.01$). Lengths of individual fish fluctuated as much as ±10% between each of 10 successive measurement dates.

Discussion

Comparisons between effects of fixation and preservation on young marine and freshwater fish are difficult because of differences in species, sizes, fixatives, sampling, handling, storage, and measurement techniques. Nevertheless, judging from our findings, marine and freshwater larvae may respond differently to some of these effects and, in addition, to salinity (Hay 1982). Thus, real differences in the extent of length changes may be related mainly to osmotic processes. Although there was a general tendency for short-term shrinkage of some body parts, lengths may also remain the same or increase, as we and Billy (1982) have shown. The latter study concerned 24–74 mm *Sarotherdon mossambicus*, fixed in 10% freshwater formalin and preserved in 37.5% isopropyl alcohol, and were the first results reported of no change or slight increase in the length of fixed fish. In our study, a number of species of newly hatched larvae did not change in mean total, standard, head, or yolk sac length when fixed with Davidson's B and measured sequentially during 45 d. Larger larvae of different species shrank 3–4%, mainly in snout to vent length.

The type and distribution of tissue in young fish differs according to species, and varies with growth and development (Gill et al. 1982). Such factors as tissue water content and the ratio of white to red muscle probably have some bearing on the extent of shrinkage due to fixation. Thus, if length changes occur in response to fixation, they would be expected to be differential in character, and more prevalent in small larvae, which contain relatively more water in their tissue than do larger fish (Ehrlich 1974). Also, formalin progressively dissolves skeletal calcium, so that the effects of preservation in formalin may be expected to depend upon the developmental stage of the larvae. We found differential changes, mainly in body length and depth, and trunk length, but shrinkage was not species specific. The fact that we observed more shrinkage in large than in small fish shows that our results differed from those of a wide variety of studies cited by Blaxter and Hunter (1982), in which there were shrinkages of 2–43%, but no increase in length of preserved marine clupeid larvae. Shrinkages of a large magnitude could seriously affect growth estimates.

According to Lockwood and de B. Daly (1975), Schnack and Rosenthal (1978), and Hay (1981), preservation duration can affect the amount of shrinkage in marine fish. In contrast, one of our freshwater species was not similarly affected, as medium-term change in total or snout to vent length in *C. commersoni* did not occur. Moreover, nine freshwater species preserved for more than 5 yr did not change significantly in a number of body characters (Leslie 1983). Also, there was no difference in preservative type relative to length changes over time. This differs from the results of Fowler and Smith (1983), who concluded that ethanol and formalin differently affect shrinkage of silver hake, *Merluccius bilinearis*.

The variation with time noted in the lengths of individual juvenile *E. a. vermiculatus* preserved with 10% Borealene for about 2 yr may reflect real change, effects peculiar to the preservative, measurement problems associated with relatively large young fish, or a combination of these factors. The influence of measurement error is seldom considered in studies of larval fish. Yet, measurement artifacts can mask or confound actual length changes, especially when fixation and preservation effects alone are small and generally inconsequential. If possible, mean values of lengths taken over a series of measurement intervals should be compared with the live or initial lengths, since, due in part to measurement error, length comparisons solely between any two dates can be misleading.

From these considerations, it is clear that the magnitude of length changes in young fish is governed by a complex set of factors. Rigor mortis is probably the most effective agent of length change in small, young fish, but measurement error doubtless plays a role in estimations of small lengths. Regardless of the extent of length changes, short- and medium-term effects on a cross-section of species of various sizes and with different body forms, i.e. short, medium, and long intestine, were of no practical consequence, and were independent of preservative type.

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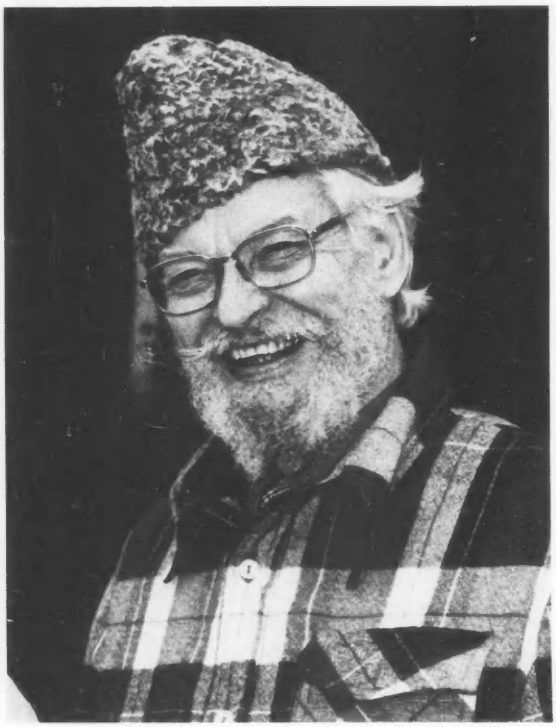
OBITUARY/NÉCROLOGIE

Paul Frederick Elson
1910–1985



With the death of Dr. Paul F. Elson on October 29, 1985, Canada lost one of its well-known fisheries research scientists, who had been associated with Atlantic Salmon investigations for most of his life.

Dr. Elson was born on December 14, 1910, in Chengtu, Szechuan, China, the son of Reverend J. Albert and Evelyn Elson, missionaries of the Methodist church, who did not return to Canada until 16 years after Paul was born.

After finishing his high school education in Ontario, Paul attended the University of Western Ontario in London, Ontario, where he received a Bachelor of Arts degree in 1934, and a Master of Arts degree in 1936, with a thesis on the ecology of the Brewers Mole. In 1936 he moved to Toronto with his wife, Elsie M. Keffer, and enrolled for graduate studies in the Department of Zoology, University of Toronto. Paul's graduate courses and Ph.D. thesis, leading to his degree in 1939, were supervised by Dr. A. G. Huntsman, head of the Marine Biology section. His association with University staff specialists in various aspects of experimental biology, including Dr. K. C. Fisher and Dr. F. E. J. Fry, provided valuable background for later extensive field work on Atlantic salmon production in a natural environment.

Paul was involved, either in an active or advisory capacity, in the federal government's field and laboratory studies of Atlantic salmon that were undertaken to elucidate varied production problems. For example, he worked for several years with Mr. Harley C. White, federal fisheries biologist and talented naturalist, on the control of predators (including kingfishers and mergansers) on young salmon on the Petitcodiac River, New Brunswick, and elsewhere. After several years on the predatory bird control program he was transferred to the Atlantic Biological Station at St. Andrews, N.B. He retired from active research in 1975 but continued to live in St. Andrews.

In 1952 the aerial spraying of New Brunswick forests with insecticides was undertaken to control ravages of the spruce budworm. Paul participated in the planning of field observations to determine effects of the various insectides on salmon production, and to recommend management procedures to supplement declining stocks of young salmon that suffered heavy losses through contact with the insecticide. The earliest aerial spraying, using formulations of DDT to be applied at 0.5 lb per acre, was done from aircraft from headwaters to downstream areas. Usually all the fry-of-the-year and many presmolt parr were killed. Paul's annual attendance at Forest Protection Ltd. meetings and elsewhere where scientific data were presented led to the use of spray formulations other than DDT (such as phosphamidon) that were less harmful to fish.

Fundamental to all the Atlantic salmon field work was Paul's interest and ability in the development of suitable fish enumeration techniques. Included were (a) sampling populations by electroseining and (b) evolution of improved design of adult fish-counting fences to record movements of both ascending and descending fish and (c) to provide an estimate of the numbers of young salmon descending the river from upstream rearing areas. Of particular significance were the counting fences on the Miramichi, Pollett, and Tobique rivers in New Brunswick. Usually when such installations were planned for other rivers in eastern Canada, Paul advised on techniques of construction and operation. He kept in touch with mathematical experts at universities for assistance with handling data obtained by counting-fence and electroseining techniques, and often performed the intricate computations himself.

Paul's participation was in demand at various national and international meetings where production and utilization of Atlantic salmon and related species were the main topics under consideration. For example, in September 1958, he was the Canadian observer at the 46th statutory meeting of I.C.E.S. at

Copenhagen, and made side trips to salmon research and management centres in Ireland, Scotland, and Sweden. Another example of his contributions internationally was the invitation to attend, with fisheries economist A. L. W. Tuomi, a meeting to consider the management of Atlantic salmon of the Foyle River System in Ireland. It resulted in a 186-page printed report by P. F. Elson and A. L. W. Tuomi, 1975, entitled "The Foyle Fisheries: A New Basis for Rational Management."

Paul's presence at the St. Andrews Biological Station is credited with encouraging the prestigious Atlantic Salmon Association to establish its international headquarters there.

Paul's interests were not all scientific, and he had several hobbies which included hunting, archery, weight-lifting, raising of goats on his small farm near St. Andrews, and perhaps most important of all, music. He had an excellent voice and for years was a member of Wesley United Church choir, and also participated in other musical activities in the town.

Besides his wife, Elsie, Paul is survived by one son, Paul of St. John, N.B.; two daughters, Mary Jo Sanger of Orono, ME, and Melinda of London, England; three sisters, Marie Elson and Joan Elson of London, Ont., and Eileen Entwistle of Cornwall, NY; one brother, John, of Montreal, Que.; seven grandchildren and several nieces and nephews.

C. J. KERSWILL
Nepean, Ontario

LETTERS AND COMMENTS/LETTRES ET COMMENTAIRES

Some Remarks on "Determinants of Catching Power in the British Columbia Salmon Purse Seine Fleet" by Hilborn and Ledbetter

In a recent paper, Hilborn and Ledbetter (1985) have presented a method for investigating factors which determine catching power in the British Columbia salmon purse seine fleet. While their conclusion that intangible factors such as skipper and crew effects probably play a large role in determining the catching power of a boat is reasonable and not surprising, there are some serious deficiencies in their methodology. In extreme examples their approach could lead to the conclusion that virtually all of the variance in catching power is explained by such intangible effects and almost none of it by general vessel attributes such as horsepower or tonnage, when the real situation is such that virtually all the variance in catch is explained by the vessel attributes. The purpose of this comment is to outline the deficiencies in their approach and to suggest a better method for analyzing their data. The solution procedure we propose can easily be carried out on a minicomputer.

Data description and notation

The data consist of catch records for about 600 boats fishing for about 15 wk a year over a 5-yr period in about eight areas. If every boat fished in one area per week for all weeks, there would be about 45 000 observations of boat catches, and we shall assume that this is the number of observations in the following discussion.

Let i index the boats, j index the areas, and k index the weeks. The observations in the model C_{ijk} are the value of the catch of boat i fishing in area j during week k. Let N be the number of boats, M be the number of areas, and K be the number of weeks fished, so that in this example $N = 600$, $M = 8$, and $K = 75$.

Model formulation

Hilborn and Ledbetter stated that the ideal model for analyzing this data would be a three-way ANOVA with interactions, i.e.

$$(1) \quad C_{ijk} = \bar{C} + V_i + A_j + W_k + (VA)_{ik} + (AW)_{ijk} + e_{ijk}$$

where the e_{ijk} are independent normally distributed random variables with means 0 and unknown equal standard deviations. They then pointed out that there are not enough observations to permit the estimation of the interaction terms. In particuliar, if each vessel fishes in one area per week, the number of parameters $(VW)_{ik}$ needed to describe the vessel−week interactions is roughly equal to the number of observations.

This is not the real point, however. The correct model is not a three-way ANOVA with interactions. Vessel−week and area−week interactions are not of interest here, although vessel−area interactions might be. The correct model to consider is 1 without the interaction terms, i.e.

$$(2) \quad C_{ijk} = \bar{C} + V_i + A_j + W_k + e_{ijk}$$

or possibly the multiplicative log-linear model:

$$(3) \quad \log_e(C_{ijk}) = \overline{\log_e(C)} + V_i + A_j + W_k + e_{ijk}$$

or the more general form used in Allen and Punsly (1984):

$$(4) \quad \log_e(C_{ijk} + c) = \overline{\log_e(C + c)} + V_i + A_j + W_k + e_{ijk}$$

where the constant c is chosen so that the log transformation produces the best "normalizing" transformation with respect to some criterion. Log-linear models have often been in fishing power analysis (Robson 1966; Gavaris 1979; Stocker and Fournier 1984).

In any event, the correct form of the model to employ can be determined by analysis of the residuals, a procedure which should always be carried out to help evaluate the results of data analysis by linear models.

If desired, the model with vessel−area interactions

$$(5) \quad C_{ijk} = \bar{C} + V_i + A_j + W_k + (VA)_{ij} + e_{ijk}$$

can also be investigated.

Since the formal difference between the additive and log-linear models given by expressions 2 and 3 is only whether the C_{ijk} or their logarithms are employed, we shall employ the notation from 2 in the following.

The point of the analysis is to determine whether there are other vessel effects explained by the parameters V_i in 2 which cannot be explained by tangible vessel attributes such as tonnage, horsepower, year built, assessed value, etc. For the conclusions drawn from analysis to be valid it is important that every effort is made to include all reasonable vessel attributes.

Assume that there are R vessel attributes T_r indexed by r. In order to include these attributes into the model it is assumed that they contribute to the catching power of the vessel in an additive fashion, i.e.

$$(6) \quad C_{ijk} = \bar{C} + \sum_r a_r T_{ir} + A_j + W_k + e_{ijk}$$

where T_{ir} is the value of the rth attribute for the ith boat. (The incorporation of vessel attributes for the log-linear model is slightly different, being multiplicative rather than additive, but this is only a small technical difference.)

The a_r are R parameters which must be estimated. Assuming that R is less than N (otherwise there is no point to the analysis), the question of the existence of intangible vessel attributes is investigated by considering the improvement in model fit, i.e. the reduction in the residual sum of squares provided by model 2 over model 6.

In Hilborn and Ledbetter there are five vessel attributes, so that $R = 5$ and the model 6 has $NK = 45\,000$ observations and $R + M + K = 88$ parameters. This can be regarded as a form of the general linear hypothesis model with a design matrix of

size 45 000 × 88. Such a model can easily be solved on a minicomputer or even on a large microcomputer with a hard disk.

Model 2 has 45 000 observations and $N + M + K = 683$ parameters. This is rather large to attempt to solve directly, although the calculations could be carried out on a large mainframe. This would be an inefficient approach, however, because the problem can be solved with considerably less computation. The parameters V_i which represent the vessel effects can easily be expressed in terms of the other unknown parameters and the problem reduced to the solution of a general linear hypothesis model containing 45 000 observations and 83 parameters which can be easily solved on a minicomputer or a large microcomputer.

Eliminating the vessel effect parameters

Let δ_{ijk} be equal to 1 if boat i fished in area j in week k and equal to 0 otherwise. The desired parameter values in model 2 are those values of the parameters which minimize the sum of squares $S(V, A, W)$ where

$$(7) \quad S(V, A, W) = \sum_i \sum_{j,k} \delta_{ijk}(C_{ijk} - \bar{C} - V_i - A_j - W_k)^2.$$

At the minimizing value the derivatives of $S(V, A, W)$ with respect to the V_i must be equal to 0, i.e.

$$(8) \quad \frac{\partial S}{\partial V_i} = -2 \sum_{j,k} \delta_{ijk}(C_{ijk} - \bar{C} - V_i - A_j - W_k) = 0.$$

Let $n_i = \sum_{j,k} \delta_{ijk}$. Expression 8 can be solved for V_i giving

$$(9) \quad V_i = \bar{C} - \frac{1}{n_i} \sum_{j,k} \delta_{ijk} C_{ijk} - \frac{1}{n_i} \sum_{j,k} \delta_{ijk} A_j - \frac{1}{n_i} \sum_{j,k} \delta_{ijk} W_k.$$

These expressions can be substituted for the V_i into the sum of squares expression 7 giving a sum of squares depending only on the parameters A_j and W_k. It has the form

$$(10) \quad S(A, W) = \sum_i \sum_{j,k} \delta_{ijk} \left[(C_{ijk} - \frac{1}{n_i} \sum_{l,m} \delta_{ilm} C_{ilm}) - (A_j - \frac{1}{n_i} \sum_{l,m} \delta_{ilm} A_{ilm}) - (W_k - \frac{1}{n_i} \sum_{l,m} \delta_{ilm} W_{ilm}) \right]^2$$

where by abuse of notation we have again employed S to denote the sum of squares.

Expression 10 is the sum of squares associated with a general linear hypothesis model. It can therefore be solved by the usual techniques, and the required reduction from 683 to 83 parameters has been demonstrated.

Expression 10 can be easily implemented in practice. Assume that the observations and the rows of the design matrix associated with 2 but without the columns associated with the vessel effects have been sorted by vessel. To obtain the design matrix associated with the sum of squares 10, it is only necessary to subtract from each element in the design matrix and the observations their average value computed over each vessel. Thus, if the data are properly sorted the reduction can be made during only one pass of the design matrix, and it is never necessary to consider the inclusion of columns for the vessel effects into the design matrix.

It should also be noted that this approach can also be applied to the model 5 which includes vessel–area interactions. It is only necessary to sort the data by vessel and area. Then, averages over each vessel–area group are subtracted from the design matrix components for that vessel–area group and one proceeds as before.

Discussion of the Hilborn Ledbetter approach

Hilborn and Ledbetter's analysis is based on the performance index P_{ij}. Define m_{ij} and p_{jk}, respectively, by

$$m_{ij} = \sum_k \delta_{ijk}$$

$$p_{jk} = \sum_i \delta_{ijk}.$$

The performance index P_{ij} is defined by

$$(11) \quad P_{ij} = \frac{1}{m_{ij}} \sum_k (\delta_{ijk}(C_{ijk} - \frac{1}{p_{jk}} \sum_q \delta_{qjk} C_{qjk})).$$

The performance index is introduced as a surrogate for the catching power in the sense that Hilborn and Ledbetter used the lack of ability of the vessel attributes to explain variation in the performance index to infer that catching power is also not well explained by vessel attributes.

In order to investigate the relationship of the performance index to the catching power, assume that the model 2 is valid. Then, performance indices satisfy the relationships

$$(12) \quad P_{ij} = V_i - \frac{1}{m_{ij}} \sum_k \delta_{ijk} \frac{1}{p_{jk}} \left(\sum_q \delta_{qjk} V_{qjk} \right) + \frac{1}{m_{ij}} \left(\sum_k \delta_{ijk} \left(e_{ijk} - \frac{1}{p_{jk}} \left(\sum_q \delta_{qjk} e_{qjk} \right) \right) \right).$$

The expected values of the P_{ij}, $E(P_{ij})$ are given by

$$(13) \quad E(P_{ij}) = V_i - \frac{1}{m_{ij}} \left(\sum_k \delta_{ijk} \frac{1}{p_{jk}} \left(\sum_q \delta_{qjk} V_{qjk} \right) \right)$$

so that as estimators of the V_i the P_{ij} will have a bias B_{ij} given by

$$(14) \quad B_{ij} = -\frac{1}{m_{ij}} \left(\sum_k \delta_{ijk} \frac{1}{p_{jk}} \left(\sum_q \delta_{qjk} V_{qjk} \right) \right).$$

This bias could be considerable, so that an inability to explain variation in the P_{ij} through vessel attributes does not imply that the same is true for variations in catching power. In addition, the B_{ij} may vary from area to area leading to between-area differences in the P_{ij} which have nothing to do with between-area differences in catching power. A simple example illustrates how use of the performance index can lead to erroneous conclusions.

Assume that there are four boats, two areas, and two time periods. In time period 1, boats 1 and 2 fish in area 1 while boats 3 and 4 fish in area 2. In time period 2, the boats are interchanged so that boats 1 and 2 fish in area 2 while boats 3 and 4 fish in area 1. Assume that there are no time–area effects and that the catching power of all the vessels is completely determined by one vessel attribute according to the relationship

$$(15) \quad C_{ijk} = 2T_i$$

and that the values of the vessel attribute for the four boats are 1, 2, 3, and 4. Now consider the performance indices for the four boats in area 1. These can easily be seen to be equal to −1, 1, −1, and 1, respectively. These are also the performance indices for area 2. When these performance indices are then regressed on the vessel attributes 1, 2, 3, and 4, only 20% of the variance in the performance indices is explained.

In testing for vessel effects, if the boats are assigned values of the vessel effects parameters of -1, 1, -1, and 1, then all the variance in the performance indices is explained. Thus, in this simple example even though all the variance in the catching power of the boats was due to a vessel attribute, the use of the performance index would lead one to conclude that at least most of the variance is due to a vessel effect.

The problem here, of course, is that there is only a poor relationship between the performance index and the catching power of the vessels. In practice the relationship between the performance index and the catching power depends in a complex fashion on which boats fish with whom, where, and at what time. It is exactly the job of a model such as that given by expression 2 to sort out this relationship to produce the estimates of relative catching power which are desired — D. A. Fournier, *Ministry of Agriculture and Fisheries, Fisheries Research Centre, P.O. Box 297, Wellington, New Zealand.*

(J8292a)

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Response to Fournier

Fournier raises a number of questions about the methods we used in our paper that apply to any attempt to determine catching power of fishing vessels. There are two major problems with his criticism; firstly, due to lack of knowledge of the fishery he has grossly underestimated the size of the problem and the subsequent design matrix. More importantly, the method he proposes for reducing the problem to a manageable size is a variation on the method we used, and one that is almost certainly inferior for the specific problem we were examining. Furthermore, Fournier implies that his approach yields the same answer as the solution to the full general linear model; this is not true and is easily demonstrated.

There is a need, however, for a clarification of the alternative forms for analysis of catching power, and the relative merits of different approaches to bringing the size of the problem to manageable proportions. Fournier's comment demonstrates that we were much too brief in our paper in explaining both the size of the problem and in discussing the alternative forms of analysis.

Size of the problem

Because the abundance of salmon in different areas of British Columbia is highly variable, and the peak of the run differs from year to year and area to area, every area–week effect in the design matrix must be considered separately. This means that the area–week interaction cannot be ignored in any analysis, and the size of the problem, at a minimum, is the number of areas times the number of weeks times and number of years.

TABLE 1. Illustration of the nonorthogonality of typical vessel week–area analysis.

	Week 1		Week 2		
	Area 1	Area 2	Area 1	Area 2	Row mean
Vessel					
1	1		1		1
2	1		1		1
3	1		1		1
4	1			2	1.5
5		2		2	2
6		2		2	2
Column mean	1	2	1	2	

This is roughly 600 elements, without any vessel attributes. Even ignoring vessel–area interaction, which we demonstrated to be significant in our paper, a linear model with 600 elements requires a large amount of time on a mainframe computer, and at the time we did our work this was prohibitively expensive.

A model that includes individual vessel effects would therefore be on the order of 600 time–area parameters and 600 vessel parameters, which is even more difficult to deal with. The time required to solve the linear model for such problems is essentially the time required to invert a matrix with order of the number of parameters. Since this matrix will be very sparse, efficient computer programs might be able to handle such large problems, but I have not had access to any.

Determining vessel effects

Fournier proposes a method for reducing the size of the models by sorting the data by vessel, and then substracting the vessel means from the catch for each vessel and eliminating the columns associated with vessels from the design matrix. He shows this by a little hand waving in solving for V_i from his equation 6, although V_i does not appear in 6 at all. However, what he really does is use the methods of two-way analysis of variance on the linear model which is exactly what we did. In fact, what we did is totally analogous to his elimination of vessel effects. We sorted our data by space–time cell and subtracted the mean catch for each space–time cell from all observations for that cell. Fournier's equation 10 could presumably be written with the space–time cells instead of the vessel effects eliminated. What we and Fournier have done is eliminate either the row or column means. The essential problem, as stated in our paper, is that the data produce a highly nonorthogonal experimental design, since each vessel does not fish in each space–time cell. Therefore, there is no unambiguous way to analyse the data.

Table 1 shows an exaggerated example in which there are six vessels, two weeks, and two areas. I have assumed that all vessels are equal, but area 1 produces twice the catch of area 2. We can eliminate the column means by sorting on week–area cells and discover that there is no vessel effect at all. However, we could alternatively sort on vessels, remove vessel effects (row means), and discover that there are hardly any week–area effects. This is our interpretation of Fournier's method, and the resulting ANOVA table is shown in Table 2.

Real data sets will not be so badly nonorthogonal as the illustration in Table 1, but how much of the variation one assigns to vessel effect will depend on the order of calculations.

TABLE 2. Analysis of variance resulting from using Fournier's proposed method on the data in Table 1. Note that the vessel effect appears to be highly significant and the area–week effect appears to be nonsignificant.

Source	SS	df	MS	F
Vessel	2.42	5	0.48	4.00
Area–week	0.15	3	0.05	0.42
Residual	0.35	3	0.12	
Total	2.92	11		

Fournier's method, which removes vessel effect first, will assign the maximum amount of variation to vessel effect, while our method will assign the minimum amount. The optimum way to deal with the problems is with the full design matrix, but as discussed earlier, this is too large for our computational facilities. I stress again that Fournier's method does not produce the same answer as the full general linear model.

However, even when the full design matrix can be solved, the partitioning of the sum of squares must be done sequentially, putting in either vessel effects or week–area effects first. The generally accepted procedure is to treat the process like a stepwise multiple regression, adding in the most significant terms until additional terms are nonsignificant.

We chose to remove the week–area effects first because of our knowlege of the British Columbia salmon purse seine fishery. There are good weeks and bad weeks of fishing for individual fishermen, and the catch per vessel will depend on how many fish there are, how many vessels there are, and the catching power of the vessels. The seine boats fish at discrete locations, and there is almost always a line-up of vessels waiting to fish desired locations. A typical seine boat spends only 10% of its time actually fishing during the hours when fishing is permitted; the rest is spent waiting or moving. Thus, we have no doubt that the dominant determinant of catch per vessel is how many vessels are present, followed closely by the abundance of fish. The only way catching power of individual vessels would affect the week–area means would be if there were large differences in catching power between vessels and the mix of catching powers between areas were nonrandom. Fournier's illustration is the extreme example of such a situation.

There are consistent differences in catch per boat between areas, as we demonstrated in our earlier paper (Hilborn and Ledbetter 1979). The two main purse seining areas, Johnstone Strait and Juan de Fuca, have consistently different catches, with Juan de Fuca usually returning 40% higher catch rates. Vessels fishing Juan de Fuca are typically larger than those in Johnstone Strait, so that using Fournier's approach we would end up assigning most of the difference between catch rates in the two areas to the longer vessels. However, when one examines the performance in either area alone, as we did in our paper, we find that vessel attributes such as length have very little to do with catch. Further, we demonstrated that vessels that perform well in one area will often not perform well in the other.

Although our method will tend to underestimate the vessel effects, Fournier's approach would much more seriously tend to overestimate them, depending on how vessels move between fisheries and the catch rates in the individual fisheries.

Suggested approaches

If the problem is not too large, the full general linear model with requisite interaction terms would be appropriate. In the following section we summarize the general order of the problem and different approaches that can be used to reduce it, including both those Fournier suggested and those we used in our paper.

Vessel attributes—The smallest problem is one where we attempt to examine the relationship between a number of vessel attributes and the catching power. No interactions between vessel attributes and area are considered. The model can be written

$$(1)\quad C_{ijk} = \bar{C} + \sum_r a_r T_{ir} + ST_{jk} + e_{ijk}$$

where C is the catch, a is a coefficient for each vessel attribute, T_{ir} is the value of the rth attribute for the ith vessel, ST_{jk} is the space–time effect for the jth location and the kth time, and e_{ijk} is the residual. If we assume there are n_i vessels, n_j areas, n_k times, and n_r attributes, then the problem involves $n_r + n_j n_k$ parameters. For the British Columbia salmon purse seine fleet this would have been 605 parameters.

There are several ways to reduce the size of the problem. First, one could eliminate marginal fishing areas and fishing weeks. If we were to concentrate on the two major purse seine areas, Johnstone Strait and Juan de Fuca, and use only the weeks each year with large fisheries, we might end up with 8 wk times 5 yr times 2 areas which is 80 space–time cells. Even using 5 or 10 vessel attributes this would still make a rather simple problem to be solved. The major problem, of course, is that we are ignoring area–gear interactions, which are discussed later.

One often has many different vessel attributes which are frequently highly correlated. One attempt at reduction, which we suggest, is a principal components analysis on the vessel attributes using only the first two or three principal components as attributes. This would have the disadvantage of making the coefficients of the model very difficult to interpret, but would likely be the best way to determine how much variation is due to vessel attributes.

For some fisheries there may be interactions between vessel attributes and areas. We demonstrated in our previous paper (Hilborn and Ledbetter 1985) that vessel attributes such as length and tonnage are more important in Juan de Fuca than in Johnstone Strait. The model with area interaction can be written as

$$(2)\quad C_{ijk} = \bar{C} + \sum_{r,j} a_{rj} T_{ir} + ST_{jk} + e_{ijk}$$

where a is now subscripted by both attribute (r) and area (j). This adds $(n_j - 1)\, n_r$ parameters to the analysis, which is not likely to be a major difficulty. For our analysis, if we used five attributes and only two areas, it would add only five additional parameters to be estimated.

Vessel effects—In our paper we were unable to explain much of the variation in differences between vessels using attributes, so we examined individual vessel differences. The general model would be of the form

$$(3)\quad C_{ijk} = \bar{C} + V_i + ST_{jk} + e_{ijk}$$

where V_i is the vessel effect. The size of this problem is $n_i + n_j n_k$ or roughly 600 + 600.

We can suggest two approaches to the problem, one we believe is possibly superior to either the method we used or the

one Fournier suggests. If we eliminate marginal space and time locations, we can reduce the number of space time cells to approximately 80 as described above. This would also eliminate some of the vessels, for instance those that never fish in Juan de Fuca or Johnstone Strait. We can then randomly choose perhaps 100 vessels and discard all others. This will leave us with 100 + 80 parameters for the full design, a solvable problem on most computers. We can repeat the random selection process a number of times and see how sensitive the results are to random selection of vessels. This procedure is somewhat similar to the bootstrap method for determining confidence limits (Efron 1979), but will underestimate the significance of the tests because we are dealing with a subset of the total data and consequently reduced degrees of freedom. There may be literature on this type of procedure for reducing the size of experimental design matrices, but given my current remote location I have not found it.

The second approach would be to first calculate the total sum of squares from the full model of equation 3, and then use Fournier's method to eliminate vessel effects and see how much the sum of squares is reduced. The procedure can then be repeated using the method we used to eliminate area−week effects and see how much this reduced the sum of squares. The "true" answer would lie somewhere between the two results and local knowledge will have to be used to determine which answer is most appropriate.

A vessel effect model with vessel−area interaction takes the following form:

$$(4) \quad C_{ijk} = \bar{C} + V_{ij} + ST_{jk} + e_{ijk}$$

where we now have a vessel effect for each area and vessel. The full British Columbia salmon purse seine analysis would thus have 600 × 8 + 600 parameters, a large problem by any standard. The same two methods for reducing the size of the problem that were described above for the vessel effects model without area interaction could again be employed for this analysis, although the reductions would still leave a reasonably large problem. In our paper we eliminated all but two areas and all vessels that did not fish both areas all years, and were thus left with an orthogonal two-way analysis of variance which is simply solved.

Summary

The linear models for determining catching power described above are growing in use for two reasons: desire to understand what determines individual vessel performance (Hilborn and Ledbetter 1985) and a desire to estimate standardized catch rates by area and time to follow stock trends (Allen and Punsly 1984). Current work at the South Pacific Commission employs similar models (Polacheck 1985). In most cases the problems are very large and some methods may be required to reduce their size. A systematic comparison of the methods used in Hilborn and Ledbetter (1985), the method suggested by Fournier, and the method suggested in this response should be performed. We are attempting to transfer the data used in our paper to our current computer to make this comparison. A similar comparison will probably be performed on SPC data as well. I would encourage others to do this type of analysis and see how well different methods work — Ray Hilborn, *South Pacific Commission, B.P. D-5, Noumea, CEDEX, New Caledonia.*

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My understanding of Fournier's comments and my response to them have greatly benefited from a number of discussions with Tom Polacheck.

EDITOR'S NOTE: Dr. Hilborn intends to reanalyze the data and submit a new manuscript reporting his findings. (J8292b)

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Letter to the Editor

The main focus of the points raised in the preceding exchange appears to be overly concerned with the choice of the "correct" model and the amount of computation required with such a model. In his summary, Hilborn encourages anyone who is interested in these problems to try out the various approaches suggested on their own data and see how they work. Before anyone rushes off to do so, we would like to review what has gone on here and clarify the issues raised. First we briefly outline for the benefit of the readers what it was that Hilborn and Ledbetter (1985) did in the article in question. At the same time we take the opportunity to standardize the notation used there and in the current letters. Then we discuss Fournier's comments on the original paper. Finally we offer comments of our own on the original article concerning important points missed in the preceding debate.

The main objective of Hilborn and Ledbetter (1985) was to determine the effect of individual vessels, general vessel characteristics (for example length, year built, etc.), fishing area, and time on the catching power of vessels in the salmon purse seine fishery. They proposed a linear model in the following symbolic notation to account for all the above with the exception of the vessel characteristics:

$$(1) \quad C = \bar{C} + V + A + W + VA + VW + AW,$$

that is, a three-way classification with vessels (V), area (A), and time (W) as main effects and their respective first-order interactions. In formal linear model notation we would rewrite the above as

$$(2) \quad y_{ijk} = \mu + \gamma_i + \alpha_j + \tau_k + (\gamma\alpha)_{ij} + (\gamma\tau)_{ik} + (\alpha\tau)_{jk} + \epsilon_{ijk}$$

where terms correspond to (1) by virtue of their order and y_{ijk} represents whatever is designated as the observed measure of catching power (e.g. value of catch, performance index, etc.). It is not surprising that such an ANOVA would be beyond the available software, since fitting the above model would require more degrees of freedom than there are observations unless replicates are available. Indeed, the $(\gamma\tau)_{ik}$ interaction (in addition to the main effects) would use up all the degrees of freedom, since, according to Hilborn and Ledbetter (1985), a vessel only fishes in one area each week.

Instead of using the catch of each vessel for y_{ijk} the dependent variable was defined to be the "annual performance" of the ith vessel in the jth area and is calculated as follows:

$$P_{ij} = \frac{1}{n} \sum_{k=1}^{n} (C_{ijk} - \bar{C}_{jk}) \quad \text{for year } l$$

where C_{ijk} is the dollar value of the catch made by the ith vessel in the jth area in week k, and n is the number of weeks a vessel fished in area j. Care must be made in using the equation because if n is less than the total number of weeks the fishery was open, then k will not necessarily go from 1 to n. In addition, C_{ijk} will not be defined and n will be zero if a vessel did not fish in a particular area.

Given the observational nature of the data, the number of observations available for each subclass will not be equal and for many combinations will be equal to zero. Therefore the authors restrict themselves to various subsets of the data (and $j = 2$ areas) in order to avoid empty subclasses.

The relationship between P_{ij} and vessel characteristics was examined by use of multiple regression. The effect of the remaining factors on P_{ij} was investigated by ANOVA techniques with individual vessels designated as treatments.

Although the concept of a design matrix was not discussed in Hilborn and Ledbetter (1985), it does appear in the letters preceding this paper. For the sake of completeness we describe the concept as follows. Consider the one-way analysis of variance model

$$y_{ij} = \mu + \beta_i + \epsilon_{ij} \quad i = 1, 2, 3 \; j = 1, 2, 3.$$

This can also be written in matrix notation as follows:

$$\boldsymbol{Y} = \boldsymbol{X}\beta + \epsilon$$

where $\boldsymbol{X}$ is designated the design or incidence matrix. The full matrices are written as

$$\begin{pmatrix} y_{11} \\ y_{12} \\ y_{13} \\ y_{21} \\ y_{22} \\ y_{23} \\ y_{31} \\ y_{32} \\ y_{33} \end{pmatrix} = \begin{pmatrix} 1 & 1 & 0 & 0 \\ 1 & 1 & 0 & 0 \\ 1 & 1 & 0 & 0 \\ 1 & 0 & 1 & 0 \\ 1 & 0 & 1 & 0 \\ 1 & 0 & 1 & 0 \\ 1 & 0 & 0 & 1 \\ 1 & 0 & 0 & 1 \\ 1 & 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} \mu \\ \beta_1 \\ \beta_2 \\ \beta_3 \end{pmatrix} + \begin{pmatrix} e_{11} \\ e_{12} \\ e_{13} \\ e_{21} \\ e_{22} \\ e_{23} \\ e_{31} \\ e_{32} \\ e_{33} \end{pmatrix}$$

The error sum of squares is $\Sigma_i \Sigma_j (y_{ij} - \mu - \beta_i)^2$ which in matrix notation would be $S = (\boldsymbol{Y} - \boldsymbol{X}\beta)'(\boldsymbol{Y} - \boldsymbol{X}\beta)$. Differentiating with respect to the parameters β and setting to zero to find the least squares estimates would give $\boldsymbol{X}'\boldsymbol{X}\beta = \boldsymbol{X}'\boldsymbol{Y}$.

The design matrix for this example and for the models considered here are not of full column rank, and hence, $\boldsymbol{X}'\boldsymbol{X}$ cannot be inverted to provide solutions for $\hat{\beta} = \boldsymbol{X}'\boldsymbol{X}^{-1}\boldsymbol{X}'\boldsymbol{Y}$. One can get around this problem by including the restriction $\Sigma\beta_i = 0$ in the $\boldsymbol{X}'\boldsymbol{X}$ matrix, or eliminating a column corresponding to one of the parameters in the design matrix and a row in the β parameter vector, or finally by using a generalized inverse (Searle 1971, chap. 4).

Fournier states that the purpose of his comment is to outline deficiencies in the above approach and to suggest a better method to analyze the data. In his opinion the deficiencies involve the use of the model in (2) instead of a model restricted to main effects only and the appropriateness of P_{ij} as a measure of vessel performance. The model he suggests is

$$(4) \quad y_{ijk} = \mu + \gamma_i + \alpha_j + \tau_k + \epsilon_{ijk}.$$

The designation of the main effects model in (4) as the *correct* model by Fournier is unfortunate, since it implies that it is possible to know which model that is and that there are no other possible models for analyzing the data. What we believe Fournier intended was given the observational nature of the data, empty subclasses, and questions about which interactions are important, that (4) is a simpler way of looking at the question of vessel effects.

However much easier this model seems to be, the fitting of the model and the testing of the hypotheses concerning the main effects present us with some problems. Denoting the reduction in sum of squares due to fitting all the effects in (4) as $\boldsymbol{R}(\mu, \gamma, \alpha, \tau)$, there are six orders in which the main effects can be fitted (3!), the reductions of sums of squares of which all sum to $\boldsymbol{R}(\mu, \gamma, \alpha, \tau)$ if μ is fitted first. Consider fitting the main effects in the following order: $(\mu, \gamma, \alpha, \tau)$. The terms for the reduction in sums of squares for this set are

Source:
$\boldsymbol{R}(\mu)$
$\boldsymbol{R}(\gamma/\mu)$
$\boldsymbol{R}(\alpha/\mu, \gamma)$
$\boldsymbol{R}(\tau/\mu, \gamma, \alpha)$
$\boldsymbol{Y}'\boldsymbol{Y} - \boldsymbol{R}(\mu, \gamma, \alpha, \tau) = \text{SSE}$
$\boldsymbol{Y}'\boldsymbol{Y} = \text{SST}.$

The hypothesis test of all levels of γ being equal requires $\boldsymbol{R}(\gamma/\mu, \tau, \alpha)$, which is not available from the above but which could be calculated by fitting the effects in the order $(\mu, \tau, \alpha, \gamma)$. For the salmon purse data there does not appear to be a natural or obvious order in which to enter the main effects. With three main effects it should be possible to calculate all six orders of reductions in sums of squares without too much difficulty. When there are more, a stepwise regression approach may be used and the data will decide on the order (see Searle 1971, p. 335–337, for a discussion of this). In general, when there are N main effects there will be $N!$ orderings of reductions in sums of squares. If, however, the data are orthogonal or balanced, then all orderings are equivalent.

Fournier raises an important point about the use of the vessel performance index P_{ij}. His point is contained in the example which deals with the four boats and two areas. The question of bias raised in equations 11–14 elludes us, since one would not expect that $E[P_{ij}] = \gamma_i$, (or V_i). Instead, if his model 2 is valid, we would assume that $E[P_{ij}] = \mu + \gamma_i + \alpha_j + \tau_k$ (if P_{ij}s are available for time period k).

In his example, which is extreme but nicely illustrates the problem, Fournier shows that the index is actually a measure of the deviations in the performance of individual vessels from the mean of an arbitrary group of vessels. When this index is regressed against the vessel characteristics, it appears that the true relationship has been reduced to a simple uninformative pattern. We illustrate this in Fig. 1 with the line defined as $C_{ijk} = 2T_i$ (Fournier's equation 15) and the stars are P_{ij}. In the example given by Hilborn in his response, all the P_{ij}s are equal to zero or are undefined, also showing the information loss inherent in the calculation of P_{ij}.

Fournier states that the whole point of analysis carried out by Hilborn and Ledbetter was to determine whether or not the effect due to vessels can be better determined by using vessel characteristics as covariates or by using individual vessels as treatments. Hilborn and Ledbetter did attempt to answer this

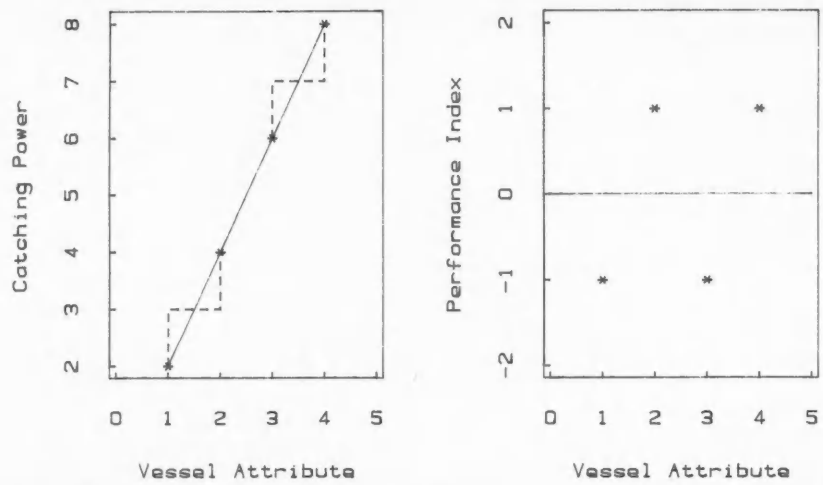

FIG. 1. Left: Plot of catching power versus vessel attributes. The four asterisks indicate the catching power for given vessel attributes in Fournier's example. The solid line shows the relationship $C_{ijk} = 2T_i$. The broken lines show how the performance index calculates the difference in catch from the mean of a group of vessels. The horizontal components of the broken lines show the means, and the vertical components show the differences between the observed catching power and the mean catching power, i.e. the performance index. Right: Plot of performance index against vessel attributes.

question although their approach and indeed that suggested by Fournier are unsatisfactory. In the former case, Hilborn and Ledbetter assumed that the reduction in their sums of squares due to a regression on the vessel characteristics is orthogonal to $\boldsymbol{R}(\mu, \gamma)$ in their table 4. Fournier, on the other hand, suggests comparing the fit of (4) with

$$(5) \qquad C_{ijk} = \mu + \sum_{a=1}^{A} \beta_a T_{ai} + \alpha_j + \tau_k + \epsilon_{ijk}$$

where T_{ai} is the value of the ath characteristic for the ith vessel. This is essentially a variation on Hilborn and Ledbetter's approach. A more satisfactory way of approaching the question would be to have a model which included both γ_i and $\Sigma_{a=1}^{A}\beta_a T_{ai}$. It would then be possible to calculate the reduction in the sum of squares due to each of these effects given that the other has been entered into the model. It is likely that this would show that $\boldsymbol{R}(\Sigma_{a=1}^{A}\beta_a T_{ai}/\mu, \gamma) < \boldsymbol{R}(\Sigma_{a=1}^{A}\beta_a T_{ai}/\mu)$, the right-hand side of which is the reduction that Hilborn and Ledbetter calculated in their regression model. The other reductions in sums of squares that could be calculated are $\boldsymbol{R}(\gamma/\mu, \Sigma_{a=1}^{A}\beta_a T_{ai})$ and $\boldsymbol{R}(\gamma/\mu)$; again the left-hand side would probably be less than the right-hand side. Calculating these sums of squares would show not only the amount of variation due to the attributes and the vessel effects, but would also clarify how dependent (or nonorthogonal) the two are. Hilborn and Ledbetter used neither $\boldsymbol{R}(\gamma/\mu, \Sigma_{a=1}^{A}\beta_a T_{ai})$ or $\boldsymbol{R}(\gamma/\mu)$ to calculate the percentages relating to "skipper-crew-net" in fig. 3, but used variance components, so they are comparing reductions in sums of squares with mean squares. This would throw their conclusions in fig. 3 into question.

As such, this form of the model assumes that there is no interaction between the covariates T_{ai} and the α_i and/or τ_k. This is equivalent to fitting a single slope $\beta_a = \beta_{ja} = \beta_{ka}\ \forall j, k$. The possibility that this could be otherwise may deserve some attention depending on the degrees of freedom available. Plots for different areas and time periods would also help.

At this point we would like to offer some comments of our own on the article by Hilborn and Ledbetter.

The first concerns what appears to be a typographical error. Comparisons of the total degrees of freedom and sums of squares between tables 3 and 4 indicate that the labelling of the fishing areas on one of these tables is incorrect.

With respect to table 4 a number of questions come to mind. First, the estimation of variance components implies that vessels were considered to be random and not fixed effects. If the vessels were treated as random effect, was a finite population correction of some sort employed? The heading for this table states that only vessels fishing all five years were included. From the degrees of freedom for vessels we understand there to be 191 vessels in the upper ANOVA providing data for each of five years. This suggests that we should have a total of 955 observations or 954 total degrees of freedom (correcting for the mean) instead of the 607 reported in the table. What has happened to the remaining 348 observations?

With respect to table 5 the authors stated that "year, vessel−year, and year−area interactions are not significant." We take this to mean that these effects were tested but from table 5 there does not appear to be sufficient degrees of freedom available. That is, to test vessel−year interaction would require 1128 degrees of freedom assuming each vessel fished all five years but there only 658 left for residuals. How were the effects tested? We cannot see how the vessel−year effect could be tested even if all the degrees of freedom were accounted for, since fitting this interaction would leave no degrees of freedom for residuals. The authors' explanation that the effects of these factors should be nonsignificant, since the averages for the P_{ij} (indexed for year?) for these factors are zero completely elludes us. This is usually the case, by definition or by restriction, in ANOVA models; and in any case, the vessel−year interaction is random if vessels are assumed to be random effects, and so we should assume the mean to be zero and test whether the *variance* of the interaction is zero.

We are concerned by the dependence of the performance index (or value of the landing) on regional variations in the price paid for the landed catches and the type of product being sold (e.g. canned, whole fresh, etc.). How much variation is there, and how likely is this to affect the data analysis?

Finally, Fournier's equations 7–10 and much of Hilborn's response is concerned with ways of making their analyses fit on their computers. That is, reducing the size of the design matrix so that $X'X$ is a smaller matrix to invert. Equations 7–9 in Fournier describe what is known as "equation absorption" (Searle 1971, p. 266). To illustrate this, consider the example given in table 1 of Hilborn's reply. We consider a main effects model of the form

$$(6)\quad C_{ijk} = \mu + \gamma_i + \alpha_j + \tau_k + \epsilon_{ijk}$$

which when written out in matrix notation would be $C = X\beta + \epsilon$. For the example in Hilborn, this would be written out in full as

$$\begin{pmatrix} C_{111} \\ C_{112} \\ C_{211} \\ C_{212} \\ C_{311} \\ C_{312} \\ C_{411} \\ C_{422} \\ C_{521} \\ C_{522} \\ C_{621} \\ C_{622} \end{pmatrix} = \begin{pmatrix} \mu & \gamma_1 & \gamma_2 & \gamma_3 & \gamma_4 & \gamma_5 & \gamma_6 & \alpha_1 & \alpha_2 & \tau_1 & \tau_2 \\ 1 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 \\ 1 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 \\ 1 & 0 & 1 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 \\ 1 & 0 & 1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 \\ 1 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 1 & 0 \\ 1 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 1 \\ 1 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 1 & 0 \\ 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 1 \\ 1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 1 & 0 \\ 1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 1 \\ 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 1 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 & 1 \end{pmatrix} \begin{pmatrix} \mu \\ \gamma_1 \\ \gamma_2 \\ \gamma_3 \\ \gamma_4 \\ \gamma_5 \\ \gamma_6 \\ \alpha_1 \\ \alpha_2 \\ \tau_1 \\ \tau_2 \end{pmatrix} + \begin{pmatrix} e_{111} \\ e_{112} \\ e_{211} \\ e_{212} \\ e_{311} \\ e_{312} \\ e_{411} \\ e_{422} \\ e_{521} \\ e_{522} \\ e_{621} \\ e_{622} \end{pmatrix}$$

In order to obtain an $X'X$ matrix of full rank, we eliminate the columns of X and the rows of β corresponding to γ_6, α_2, and τ_2 (there are other possibilities, but this is the most common procedure). Least squares estimation would give the normal equations $X'X\beta = X'C$. Fournier absorbs the γ_i into the α_j and τ_k. In this example, this is accomplished by absorbing the second to sixth normal equations into the others. By writing the normal equations out in full as in the previous example, we obtain

$$\begin{aligned}
\gamma_1 &= \bar{C}_{1..} - \mu - \alpha_1 \quad -\tau_1/2 \\
\gamma_2 &= \bar{C}_{2..} - \mu - \alpha_1 \quad -\tau_1/2 \\
\gamma_3 &= \bar{C}_{3..} - \mu - \alpha_1 \quad -\tau_1/2 \\
\gamma_4 &= \bar{C}_{4..} - \mu - \alpha_1/2 - \tau_1/2 \\
\gamma_5 &= \bar{C}_{5..} - \mu - 0 \quad -\tau_1/2
\end{aligned}$$

which gives the same result as Fournier's equation 9, where his $\Sigma_{j,k}\delta_{ijk}$ is equal to the numbers preceding the parameters in the $(i + 1)$th normal equation. It also has the same interpretation as that given by Fournier. We could simplify the notation by just saying that

$$(7)\quad \gamma_i = \bar{C}_{i..} - \mu - f_i(\alpha_1, \tau_1).$$

Fournier then takes equation (7) and substitutes it back into equation (6), that is

$$C_{ijk} - \bar{C}_{i..} = -f_i(\alpha_1, \tau_1) + \alpha_j + \tau_k + e_{ijk}.$$

What we have achieved is to replace γ_i with a function of α_1 and τ_1 which still depends on i. When these reexpressions of γ_i are inserted into the remaining normal equations, we are left with as many equations in μ, α, and τ as we have parameters to estimate; thus, estimations can now be done by inverting a 3×3 matrix instead of the original 8×8 matrix. Once these parameters are estimated, the γ_i are then estimated by equation (7). This is necessary in order to calculate the reductions in sums of squares required for tests. We have therefore not reduced the number of parameters to be estimated, but we have estimated them by different methods, and in particular we have reduced the size of the matrix to be inverted. If there were 600 vessel effects, two areas, and five time periods, for example, we could "sweep" out the vessel effects and be left with a 6×6 matrix instead of a 605×605 matrix to invert. Fournier's use of equation 10 is unnecessary and Fournier does not make clear exactly what the sum of squares is that he is trying to minimize.

For more details on this we refer the reader to Searle (1971). It is our opinion that this method (which is available in the SAS statistics package (SAS Institute Inc. 1982)) would be very useful for the types of problems Hilborn discusses.

We conclude by presenting some useful principles of (statistical) modelling given in McCullagh and Nelder (1983).

(1) All models are wrong; however, some are better than others. There is no justification given in Hilborn and Ledbetter (1985) for assuming that a linear additive model is "best." Fournier's suggestion of an alternative multiplicative model is well taken. There is also no justification for saying that a model is "correct."

(2) Application of more than one type of statistical model to the data may offer different and interesting insights. The data may point equally well to several possible models. It is rare that two data analysts will come up with the same model for the same set of data. A good example of this is shown in Whitmore and Gentleman (1985), where two analysts (Moore and Zwiers) were each asked to analyse the same set of data.

(3) Always check the fit of the current model through residuals and other diagnostics. Again, Fournier's point about checking residuals is well taken — S. J. Smith, *Marine Fish Division, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, N.S. B2Y 4A2*, and Phillip E. J. Green, *Consulting Statistician, Department of Mathematics, Statistics and Computing Science, Dalhousie University, Halifax, N.S. B3H 4H4.* (J8515)

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In Defense of the Instream Flow Incremental Methodology

Mathur et al. (1985) recently criticized the Instream Flow Incremental Methodology (IFIM), a technique for recommending flows for streams, on the basis of the evidence available to support the main assumptions. The major point of their article was that the critical assumption of a positive relationship between weighted usable area (WUA), a measure of usable habitat, and biomass of fish was not well established. While this concern and others expressed by Mathur et al. (1985) are legitimate and should be addressed in continuing research and methodology development, the article contained several misinterpretations of previously published results. Our comments deal with three topics: (1) suitability index curves, (2) habitat–biomass relationships, and (3) validation studies.

The suitability curves depicted in fig. 1 of Mathur et al. (1985) were used to demonstrate that curves developed from different streams can vary in shape and location. However, the curves shown were developed using only habitat utilization data and adjustments were not made for habitat availability at time of sampling. Baldridge and Amos (1982) and Orth et al. (1982) demonstrated the importance of adjusting for habitat availability at time of sampling. Suitability curves based on only utilization data will usually be biased.

A positive relationship between WUA and fish biomass or numbers is assumed in any IFIM application. The evidence to support this relationship indicates that the relationship holds only during periods of limiting habitat. Furthermore, time lags between habitat limitation and population response complicate interpretation of the nature of the relationship in unregulated streams. Mathur et al. (1985) plotted our data (Orth and Maughan 1982) on seasonal biomass and WUA in their fig. 3 and 4 to support their conclusion that no positive, linear relationship exists. Their plots are misleading, since data from all seasons were pooled; each plotted datum represents a weighted average from four sites (two pools and two riffles). Furthermore, one datum in fig. 4 (freckled madtom; 199 m^2, 2 g) was not even in our data set. It was unreasonable of these authors to expect any relationship when data were plotted in this manner. Our hypothesis has been that usable habitat may limit population biomass only during limited time periods. While this can occur in any season, we found that biomass of three riffle-dwelling fish species was correlated with their respective measures of WUA for the summer only (Orth and Maughan 1982). In winter and spring, WUA was usually higher but fish populations did not respond because recruitment of young fish had already been limited by summer conditions. This hypothesis was later supported by Loar et al. (1985) who found that brown trout (*Salmo trutta*) biomass was correlated with the minimum WUA for adults over the year ($R^2 = 0.71$; $P = 0.0001$) and rainbow trout (*Salmo gairdneri*) numbers in allopatric populations were correlated with the minimum WUA for incubation ($R^2 = 0.62$; $P = 0.0001$). These results emphasize that available habitat can limit fish populations during relatively short time periods. However, available habitat is not always positively related to population abundance due to other potential limiting influences, such as interspecific competition (Loar et al. 1985). These complicating factors do not detract from the usefulness of IFIM but they do point to the need for more research on the influence of stream flow on stream fish populations and communities.

To rigorously test IFIM, one would need to use IFIM to determine stream flow regimes to meet specified objectives, implement the recommended flow, and measure standing stocks of fish to see if they responded in the manner predicted. No such methodology validation studies have yet been conducted and this will continue to limit the acceptability of IFIM among scientists and water resource managers. Mathur et al. (1985) cited the study by Kraft (1972) as evidence that reduction in summer flows altered only distribution and not abundance of brook trout (*Salvelinus fontinalis*). However, in Kraft's (1972) study, changes in physical characteristics of the study sections after a 90% reduction in flow were not necessarily detrimental because the study stream had a well-defined channel and the reductions in available habitat were not in proportion to flow reductions. Since availability of usable area was not quantified, it was wrong to conclude, as Mathur et al. (1985) did, that usable area may not have a regulatory effect on the population. In conclusion, while we agree that further study is needed, we support the current practice of making the assumption that WUA is an index of potential fish biomass or numbers — Donald J. Orth, *Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA*, and O. Eugene Maughan, *Oklahoma Cooperative Fish and Wildlife Research Unit, Oklahoma State University, Stillwater, OK 74078, USA.* (J8441a)

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Reply to "In Defense of the Instream Flow Incremental Methodology" by D. J. Orth and O. E. Maughan

Orth and Maughan's comments on our paper (Mathur et al. 1985) provide further evidence that application of the Instream Flow Incremental Methodology (IFIM) in its present form should be abandoned. As stressed in our paper, the requirement for a positive linear correlation between weighted usable area (WUA) and fish biomass is essential. Their comments still fail to address this and other central issues.

The original assumption of IFIM is that usable habitat expressed in WUA (absolute units) is linearly and positively related to fish biomass (absolute weight). WUA is roughly equated to the carrying capacity of a stream. Therefore, the only relevant test of that assumption is the correlation between the biomass of each life stage of fish and its calculated WUA.

In defense of IFIM, Orth and Maughan selectively cited the results of Loar et al. (1985). Of the 160 possible correlations for the rainbow trout, *Salmo gairdneri*, only 14 (9%) were significant. None of the significant correlations were between WUA and fish biomass.

Negative correlations were also obtained by Loar et al. (1985). It is puzzling that Orth and Maughan highlight a minority of significant positive relationships (even though irrelevant to the basic assumption) and avoid even mentioning the overwhelming number of nonsignificant or negative relationships reported by Loar et al. (1985). Negative and weak correlations were also reported by Shirvell and Morantz (1983) for four years of IFIM data on juvenile Atlantic salmon, *Salmo salar*. A flow diversion study using IFIM by Irvine (1984) provided similar results.

Recently, Scott and Shirvell (1986) examined all the statistical correlations between usable habitat and fish biomass reported in the literature. Of the 517 correlations, 82% were nonsignificant; of the remainder, some were positive and some negative. Because a positive linear relationship is found so rarely, it is reasonable to conclude that such relationships are really due to chance and WUA can not be considered a predictor of fish biomass. In light of these analyses it is hard to conceive how Orth and Maughan can continue to have faith in WUA.

Orth and Maughan commented on the presence of correlation between biomass of three riffle dwelling species and their respective percent WUA during the summertime in Glover Creek. We interpreted their data (Mathur et al. 1985) to indicate merely seasonal variations in biomass independent of changes in WUA. We again do so. We believe their argument that limited recruitment of young fish occurred in summer is illogical with respect to the data and contradictory to their statements. Examination of their data in tables 5 and 6 in the time sequence they were collected, i.e. beginning in fall (November–December) 1977 to summer (August–September) 1979, reveals that the biomass of all the riffle dwelling species was higher in spring and summer 1979 following the near zero flows experienced in summer–fall 1978. Where did these fish come from if the population was being limited or recruitment was restricted in summer–fall? WUA provides no explanation.

The failure of proponents of IFIM to distinguish between changes in distribution of fishes and abundance continues to lead to misinterpretation of the available data. No study shows homogenous distribution of fish population over time and space in any body of water. There is always a "decline" (redistribution) in fishes from summer to winter at a given location. The fact is that distribution of stream fishes changes through seasons. Such was the case in Glover Creek.

Orth and Maughan believed that it was unreasonable for us to plot their pooled data in tables 5 and 6 (two pools plus two riffles over seasons and sites). This is a tenuous and strange contention. Data plotted in our fig. 3 and 4 were simply those given by Orth and Maughan in their tables 5 and 6. Presentation of identical data in different format should not logically lead to different conclusions. They used those pooled data as a basis for concluding whether or not usable habitat was limiting abundance of fishes in Glover Creek (Orth and Maughan 1982, p. 427, 429–431). The seasonal statistical correlations between percent WUA and fish biomass (kilograms per hectare) presented by them were based on analysis of pooled data (riffles, pools, and years) from two years. Our reanalysis showed those correlations were spurious. Their reported relationships were also based on suitability curves obtained for one season and one year and applied to the entire data set from two years and all seasons. No evidence exists in the literature which supports the view that such a generalized curve is sufficient to describe microhabitats of fishes, particularly when the seasonal and daily changes in behavior of fishes are considered.

Orth and Maughan's comment that the "suitability" curves developed after adjustments for habitat availability are unbiased shows only that they did not critically evaluate the implications of the curves presented by Mathur et al. (1985). First, no evidence in the primary literature demonstrates that adjustment in suitability curves for the availability of the three physical habitat attributes (as measured in IFIM) will improve the prediction of biomass of fishes. Second, we showed that such curves will differ with streams, size, stream flow, and seasons and also over a 24-h period, thus causing large biases in calculation of WUA. Larimore and Garrels (1985) provided examples of large diurnal differences in suitability curves for the northern hogsucker, *Hypentilium nigricans*, in the same stream. Bain et al. (1982) cited the differences in suitability curves for the smallmouth bass, *Micropterus dolomieui* (both juvenile and adults), at different discharges and seasons within the same river. Third, no uniform criteria exists on where to measure the distribution of depths and velocities at different flows to obtain information on availability and utilization of physical variables. An investigator can get any number of WUA estimates he wishes depending upon which curves (day, night, or season, etc.) he selects for the calculations.

Orth and Maughan reject our interpretation of Kraft's (1972) study as showing that biomass and flow are unrelated because the study stream had a well-defined channel and the reductions in available habitat were not in proportion to flow reductions (the summer flow was reduced by 90%). This contention is absurd because Kraft (1972) noted that depth, velocity, and

surface area changed significantly with the flow reductions. These physical variables are integral components of WUA. Kraft's results cannot be rejected lightly in face of the other supporting evidence on redistribution of fishes when stream flow is altered or significantly reduced (Clothier 1954; Rimmer 1985). The major conclusion to be drawn is that fishes do not respond as IFIM predicts.

One source of confusion in the interpretation and application of IFIM is the method of manipulation of the data to satisfy assumptions. As indicated earlier it was originally assumed that WUA is approximately equivalent to carrying capacity and can be used as a surrogate for it. As such, relating biomass to WUA is actually equivalent of plotting "*X*" versus "*X*". Such a plot should yield a slope of 1; this never occurs. However, when WUA is expressed as a percentage (a dimensionless number) of the total area, it has no physical meaning. It is puzzling to us why the relationship between percent WUA and biomass of fish (kilograms per hectare or kilograms per WUA) is tested to satisfy an IFIM assumption and then a flow recommendation is made from the relationship of actual stream flows versus WUA. If WUA is related to the stream flow and fish biomass, then why not examine the relationship between natural river flows and fish biomass and get the desired result directly? We can only speculate on the possible reasons. Most correlations reported in the literature relating biomass or abundance of fish and natural river flows are negative. A negative relationship, although predictive in many cases, is not intuitively palatable to IFIM supporters because it implies reduced flows for greater fish biomass.

We support in theory the proposal by Orth and Maughan to do a validation study of IFIM but believe that it may be difficult or impossible to validate for reasons given earlier (Mathur et al. 1985).

Since our original paper, recent publications provide evidence that the IFG-4 hydraulic simulation model (a component of IFIM) is not reliable for applications in many streams (Shirvell and Morantz 1983; Shirvell 1984; Loar et al. 1985). The errors or biases introduced via hydraulic simulations coupled with the unreliability of the WUA index in predicting fish biomass can only lead to compounding of errors and confusion.

From the above discussion we conclude that IFIM in its present form is more about "perceptions" or "potentials" rather than the reality of population dynamics and natural history of fishes. The fisheries profession should not be asked to accept a relationship (of potential habitat and population size) on blind faith. Orth and Maughan offer us nothing more — Dilip Mathur, *RMC Environmental Services, Muddy Run Ecological Laboratory, 1921 River Road, P.O. Box 10, Drumore, PA 17518, USA*, William H. Bason, *Delmarva Ecological Laboratory, Inc., R.D. 1, Box 286, Middletown, DE 19709, USA*, Edmund J. Purdy, Jr., *Philadelphia Electric Company, 2301 Market Street, Philadelphia, PA 19101, USA*, and Carl A. Silver, *Department of Statistics, Drexel University, Philadelphia, PA 19101, USA.* (J8441b)

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BOOK REVIEWS / COMPTES RENDUS

FAO species catalogue. Vol. 4. Sharks of the world: an annotated and illustrated catalogue of shark species known to date. *L. J. V. Compagno. Food and Agriculture Organization of the United Nations, Rome, Italy. In 2 parts, Part 1: 1984, p. 1–250; part 2: 1985 (1984), 251–655.*

To date there have been only two books that have attempted to review all known shark species: Müller and Henle's "Systematische Beschreibung der Plagiostomen" (1838–1841) and Garman's "The Plagiostomia" (1913). Since so much has been learned about sharks since 1913, a book like Compagno's has long been awaited. So much research on sharks is going on (in 1985 there were four international shark symposia, in Baltimore, Knoxville, Tokyo, and Corvallis, three of which will have published proceedings) that much of this book was out of date by the time it was published. Up to 1983 this work adequately covers the taxonomic and biological literature of sharks.

Despite many problems, this work incorporates and organizes a monumental amount of information on the taxonomy and biology of sharks. It will serve as a popular general reference on sharks for years to come. But for serious scientific research the following caveat is issued — let the user beware.

Almost every species accepted herein as valid is illustrated. The drawings vary from simple outlines to beautiful, detailed stipple drawings, and in quality range from very poor to excellent. It is unfortunate that they could not all have been drawn to the same uniform standard of excellence. All keys to families and genera are accompanied by useful diagrams of key characters. Unfortunately, the keys to species are not accompanied by similar diagnostic diagrams. The figures are numbered idiosyncratically: individual species illustrations are unnumbered, but key figures in each section are numbered in separate series. Part 1, therefore, has 15 figures 1 and part 2 has seven figures 1! There are some figure errors, e.g. on p. 33 there are two figures 25b and no figure 25a; on p. 482 the figure of *Carcharhinus limbatus* clearly has black on the anal fin, whereas the text says the species rarely has a black spot on the anal fin (a character used to separate most *C. limbatus* from *C. brevipinna*); on p. 331, one figure for *Halaelurus quagga* says "underside of head," but it is clearly a dorsal view; and on p. 522 the figure of *Prionace glauca* teeth is printed upside down, as are the teeth of *C. leucas* on p. 479.

Both parts are marred by many typographical errors and omissions, and part 2 has several pages of corrections for part 1, which the reader should add in before attempting to use the volume. Even after these corrections are made, there are still many errors. A major problem is the generic and specific name misspellings: *Oxinotus* for *Oxynotus* Rafinesque on p. 24; *Crossohrinus* for *Crossorhinus* Bleeker on p. 179; *Synondontaspis* for *Synodontaspis* White on p. 215; *Lamliostoma* as in *Lamiostoma belyaevi* Glikman on p. 244; *Atelomycterius* for *Ateleomycterus* Fowler on p. 292; *Carcharodon carcharis* for *C. carcharias* (Linnaeus) on p. 103; *Squalus cornumicus* for *S. cornubicus* Bloch and Schneider on p. 246; and *Parmaturus campechensis* for *P. campechiensis* Springer on p. 340.

Some of the numerous errors and omissions must be discussed or corrected here. On p. 111, authorship of *Squalus acanthias* is attributed to Smith and Radcliffe, 1912, whereas it should be Linnaeus, 1758. On p. 66, parentheses are missing around Garman, 1906 in the authorship of *Deania histricosa*. On p. 167 and 168, the authorship of *Cirrhoscyllium* and the species *C. expolitum* should be Smith and Radcliffe, *in* Smith, 1913. On p. 180, *Sutorectus* Whitley is placed in the synonymy of *Orectolobus* Bonaparte, but *Sutorectus* is recognized as a valid genus on p. 186. On p. 191, for *Chiloscyllium griseum* a holotype is listed at Muséum National d'Histoire Naturelle, whereas in reality there is a syntypic series of seven specimens. On p. 257, *Pentanchus* Smith and Radcliffe is listed as a synonym of *Apristurus* Garman, but it is treated as a valid genus on p. 345. On p. 261, the date of authorship is cited as 1932 for Koefoed, 1927. On p. 300, *Cephaloscyllium nascione* is attributed to Whitley, 1932 who described *nascione* as a form of *C. isabella isabella laticeps*. According to the International Code of Zoological Nomenclature, this has the rank of an infrasubspecies, and, as such, a form takes authorship only when it is elevated to subspecies or species status. Thus, this species should be listed as *C. nascione* Springer, 1979, in as much as he elevated it to a subspecies of *C. laticeps*. On p. 343, under *Parmaturus pilosus* it is stated that Springer (1979) examined the holotype (now lost) of the synonymized *P. hertwigi*, but Springer (1979) clearly stated that the holotype had been lost during World War II and that his synonymization is based on data "in Engelhardt's description." On p. 358–359, under *Scyliorhinus canicula* (Linnaeus), the frequent erroneous use of *Scyliorhinus caniculus* is not mentioned (*canicula* is a proper feminine noun in apposition). Cadenat and Blache (1981) are not cited or discussed with respect to the questionable West African records for this species; nor, on p. 361, is their synonymization of *Scyliorhinus cervignoni* Maurin and Bonnet with *S. stellaris* (Linnaeus) cited or discussed. On p. 376, *Proscyllium* Hilgendorf: this genus was proposed for the species *habereri*, not *venustum*. On p. 440, the genus *Hemipristis* Agassiz is feminine and the adjectival species name should be *Hemipristis elongata* (Klunzinger). On p. 505, female *Galeocerdo cuvieri* are stated to be longer than 55 m, which probably should read 5.5 m. On p. 525, under *Rhizoprionodon acutus* Rüppell, it is stated that alternative spellings for synonymous names have also been *walbenii* and *sorrakowah*. As done in other sections, they should be listed with authorships and dates. On p. 541, in the synonymy of *Sphyrna* Rafinesque, it should be stated that *Zygaena* Cuvier, 1817 is also preoccupied by *Zygaena* Fabricius, 1775 in Insecta. In the prefaces of both parts it is stated that many illustrations were redrawn from Okutani (1980), which is missing from the bibliography.

On the whole, the keys to families, genera, and species are useable. There are, however, many relative characters which will be difficult, e.g. "snout shorter" versus "snout longer" (p. 16, couplet 2); "eyes very large" versus "eyes small" (p. 16–17, couplet 3); "mouth long and more narrowly arched" versus "mouth short and more broadly arched" (p. 47, couplet 4). Errors in the keys complicate their use: p. 336,

couplets 1a and 1b both say "3½ times its height or less"; p. 453, couplet 19a and 19b, the parts dealing with internarial space compared with preoral length seem to have been interposed, in as much as couplet 19b is otherwise in conflict with couplet 28 below. A key to the orders of sharks is lacking, unless one uses the diagram in figure 1 on p. 3 as a key. However, the character "mouth behind front of eyes" does not separate many scyliorhinids from the Orectolobiformes.

The sections devoted to diagnostic features for each taxa are really just general descriptions, including much excess and useless information, e.g. under *Hexanchus*, p. 19, "head broad or narrow and pointed"; under Squalidae, p. 28, "trunk stout to slender," "with or without abdominal ridges"; "caudal fin with or without a subterminal notch." For almost every squaloid genus it is stated: "cloaca not expanded as a luminous organ." On p. 14, *Chlamydoselachus* is said to exhibit "keels on abdomen"; I have never seen a *Chlamydoselachus* with keels on its abdomen.

The choice of a preferable common name is often very difficult, with either none available (so you have to make one up) or many local ones to choose from. Several of the choices in this work are questionable. For example, *Squalus acanthias* Linnaeus, which is widely dissected in high school and college biology classes, about which numerous dissection guides have been written, and which is widely known as the spiny dogfish, is here called the piked dogfish (p. 111). *Oxynotus*, and its various species, which have been called prickly sharks in many publications, are here called rough sharks (p. 124). Only English, French, and Spanish common names are provided. Since many of the species live, or are commercially important, in countries such as Japan, Taiwan, the U.S.S.R., Germany, etc., in which English, French, or Spanish are rarely if ever used, it would have been useful to include local common names in the language of that country.

In many instances the systematic information provided is questionable, erroneous, or not justified. On p. 25, regarding the Echinorhinidae, it is stated that "morphological study indicates that it is very distinct from the Squalidae," but no data are given to support this view. On p. 29: "*Oxynotus* is close to the genera placed in Somniosinae and should share a common group with them", yet on p. 124, *Oxynotus* is kept in a separate family, Oxynotidae. On p. 34 it is stated that although *Aculeola* is close to *Centroscyllium*, it is clearly distinct based on anatomical characters, none of which are mentioned; further on it states "feeding habits unknown" for *Aculeola*, but goes on to say it probably feeds on "small fishes and invertebrates," with no supporting data. On p. 40, *Centrophorus ferrugineus* Chu, *et al.* is synonymized under *C. lusitanicus* with only the simple statement "may be identical." Similarly, on p. 56, *Centroscymnus macrops* Chu, *et al.* is synonymized under *C. coelolepis* with the comment "possibly identical." On p. 140, *Squatina fimbriata* Müller and Henle is placed in the questionable synonymy of *S. aculeata*, and on p. 144, *S. philippi* (Garman) in the questionable synonymy of *S. californica*, both without comment. While working on the MCZ chondrichthyan type catalogue, K. E. Hartel and I examined the holotype of *S. philippi* and compared it with specimens of *S. californica*. In numerous characters (described elsewhere) the type is very distinct from *S. californica*, although it may be identical to some other species. This makes me question synonymies made by Compagno without comment. However, good to see that after many years of using *Eugomphodus* without formally resurrecting it, Compagno finally has revealed his reasons for using this name (p. 215). To me, these characters are not sufficiently different to justify a distinct genus, and I advocate the continued useage of the genus *Odontaspis* to include the species *taurus* and *tricuspidatus*. On p. 484, Compagno follows Garrick (1981) in using *Carcharhinus longimanus* Poey, 1861, rather than the senior synonym of *C. maou* Lesson, 1830, because *C. longimanus* was placed on the Official List of Species Names in 1965. However, the placing of the name on the List was done as part of the move to clarify exactly what species *Carcharodon carcharias* was, and the name *C. maou* never entered into that discussion (because *C. maou* was not known to be the senior synonym). To be permanently resolved, this problem must be presented to the Commission for a decision, as Garrick (1981) recognized. On p. 489, *Carcharhinus obscurella* Deng, Xiong, and Zhan, 1981, is synonymized under *C. obscurus* LeSueur without comment. On p. 500, Compagno follows Garrick (1981) in using *Carcharhinus sorrah* Valenciennes, 1839, instead of the senior synonym *C. spallanzani* Peron and LeSueur, 1822, because of the inadequacy of the latter's original description and the subsequent confusion. These are not valid reasons for abandoning the older name, unless the International Commission formally rejects it. Until this occurs, the name *C. spallanzani* Peron and LeSueur, 1822, must be used for this species. On p. 540, the genus *Eusphyra* is resurrected for the species *blochi* Cuvier, although Gilbert (1967) found no compelling reason to elevate it above a subgenus. I do not find the reasons given here any more convincing than those of Gilbert, but by separating off *Eusphyra blochi*, *Sphyrna* becomes paraphyletic.

Compagno attributes all ordinal names to himself. Although the International Code does not specifically cover ordinal names, general usage dictates that they should be treated in a manner similar to family names, that is, with the author of first ordinal-level use given the authorship, even if he (or she) did not use the -iformes ending. Thus the ordinal authorships would be as follows: Hexanchiformes White, 1936 (p. 13); Squaliformes Gill, 1862 (p. 24); Pristiophoriformes Berg, 1940 (p. 130); Heterodontiformes Berg, 1937 (p. 154); Orectolobiformes Applegate, 1972 (p. 165); Lamniformes Bertin, 1939 (p. 212); Carcharhiniformes Garman, 1913 (p. 251); and Squatiniformes Bertin, 1939 (p. 562).

In the generic synonymies there are quite a number of omissions. These include *Chlamydoselachoides* Fowler, 1947 under *Chlamydoselachus* Garman (p. 14); *Heptancus* Agassiz, 1846 under *Heptranchias* Rafinesque (p. 17); *Monopterus* Blainville, 1816, *Holodus* Agassiz, 1842, and *Notidanion* Jordan, 1923 under *Hexanchus* Rafinesque (p. 19); *Dalatius* Agassiz, 1845 under *Dalatias* Rafinesque (p. 63); *Squallus* Scopoli, 1777 under *Squalus* Linnaeus (p. 109); *Squaloraia* de la Pylie, 1835 under *Squatina* Bonaparte (p. 138) and on the same page *Squalraia* should be attributed to Fowler, 1969, not de la Pylaie; *Hannovera* Beneden, 1871 and *Tetraoras* Fowler, 1967 under *Cetorhinus* Blainville (p. 234); *Charcharodon* Cisternas, 1867 under *Carcharodon* Smith (p. 238); *Ateleomycterus* Fowler, 1928 under *Atelomycterus* Garman (p. 292); *Prionorus* Otto, 1821 under *Galeus* Rafinesque (p. 306); *Catulus* Valmont, 1769 under *Scyliorhinus* Blainville, 1816 (p. 355) (this does not, however, change the valid generic name, as *Catulus* is preoccupied by *Catulus* Kniphof, 1759 in Insecta); *Scylium* Eichwald, 1819 under *Scyliorhinus* Blainville (p. 355) (misspelled *Scyllium*); *Cynais* Fowler, 1908 under *Mustelus* Link (p. 397); *Isoplagiodus* Gill, 1862, and *Charchrinus* Jordan and Starks, 1917 under *Carcharhinus* Blainville

(p. 449); *Galeocerda* Munro, 1955 under *Galeocerdo* Müller and Henle (p. 503); *Alopecula* Valenciennes, 1881 under *Prionace* Cantor (p. 521); and *Sphyrnichthys* Thienemann, 1828, *Sphyrichthys* Fowler, 1968, and *Euspyra* Gill, 1862 under *Sphyrna* Rafinesque (p. 541). The species synonyms *Hemiscyllium malayanum* Bleeker, 1852 and *H. malaisanum* Bleeker, 1857 are missing under *H. freycineti* (Quoy and Gaimard) (p. 196). Also, *Squatina angelina* Gray, 1851 is omitted from the *Squatina* section. Judging from the above number of generic and species synonyms omitted, I suggest that there may be more omissions that I am not aware of due to my lack of knowledge in those groups.

One drawback with the format of this opus is that for each species, the subsection on biology and the literature references are separate, so that if one wants to pursue further a particular aspect of biology, all references listed may have to be perused before the source of the desired information can be located.

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(R605)

Erie: the lake that survived. *Noel M. Burns. Rowan and Allanheld Publishers, Totowa, NJ. 1985. 320 p. $34.95 (U.S.).*

Perhaps no other degraded ecosystem in North America has made a comparable impact in the struggle for environmental awareness. The plight of Lake Erie in the 1960's laid to rest the myth that "dilution is the solution to pollution" and contributed to the slow recognition that man, through carelessness, ignorance, and greed, could adversely affect such a large body of water and its diverse biota. Reports of the U.S. Federal Water Pollution Control Administration, the International Joint Commission, and the published findings of Project Hypo alerted the public to the seriousness of Lake Erie's problems and influenced governments to implement remedial efforts.

As far as I know, this is the first attempt in North America to feature, in book length, the story of how man almost destroyed a large, important freshwater resource and how he is now attempting restoration. Part of the author's purpose in writing the book "is to ensure that the remedial program continues and grows so that Lake Erie may not only survive, but one day become a healthy ecosystem." To assist with that goal, the book must be read and understood and therefore the author has aimed for a fairly wide readership — the intelligent layman. The book may hit the mark because the subject matter is general and broad enough to be interesting and useful to members of the public concerned about man's impact on our environment, college and university students as an ecological textbook, and scientists and administrators involved with understanding degraded ecosystems and strategies for their rehabilitation.

The text is organized around 12 chapters grouped under four main themes. The first part is historical and contains three short chapters on geological development, exploration and early settlement, and a brief introduction to the changes caused by man's development of the basin. By describing the adversities faced by early settlers and the seemingly endless abundance of fish and game, the author attempts to explain the early careless attitude towards natural resources which, unfortunately, prevailed well into the twentieth century.

The second part is a description of natural processes in the lake and how they respond to loadings of nutrients, toxins, and other materials. Some of the information is basic and its inclusion stems from the author's aim to be intelligible to the nonexpert reader. Moreover, descriptions of basic physical and chemical processes are applied directly to Lake Erie. Models of some of the processes are described separately in an appendix. The author has made a genuine attempt to simplify the complex science of water movements by listing the many types of currents, circulation patterns, and waves and describing briefly their main characteristics. The chapter on loading of nutrient and nontoxic materials reflects the author's extensive knowledge and experience as a chemical limnologist. His discussion of nutrient overloading and the eutrophication process includes a brief account of the phosphorus–carbon controversy. Burns appears to be less comfortable with the subject of toxic substances, perhaps because of the paucity of information, particularly for the biota, and the lack of characterization of the flow and fate of contaminants in the food web.

About one third of the book is devoted to an in-depth discussion of the ecology of each of the three main basins of the lake. To me, this is the best part of the book. Emphasis is on historical changes as measured by nine variables or descriptors of nutrients, oxygen, plankton, and benthos. In an ecosystem approach, the author has integrated information on Lake Erie's fishes and man's effects on them through exploitation. Although not a fisheries scientist, Burns is aware of the biases in the historical data. He also recognizes that the array of stresses acting on the Lake Erie fish community make delineation of effects of environmental perturbations difficult. Much of the chapter on the central basin is concerned with research and includes an excellent summary of Project Hypo which was a 1970 investigation into the problem of oxygen depletion in the summer hypolimnion. Burns is at home here because he was a project leader and has since followed his early work with additional studies and analysis. Project Hypo was a key study in the Lake Erie story and its findings influenced directly the recommendations for phosphorus limitations in the 1972 *Great Lakes Water Quality Agreement*.

In the final part of the book, the author summarizes current problems, remedial efforts to date, and what remains undone. He is optimistic about the future of the lake but also practical about how far back along the degradation curve our rehabilitative efforts will take us. He warns that increasing inputs of energy, funding, and innovation will be needed if ecosystem quality is expected to keep ahead of perturbations from an expanding population.

The production of the text is good. Most of the illustrations and photographs, which are numerous, are clear. Typographical and other errors are few, but obvious when located in figure captions.

In my view, Burns has achieved his purpose in writing this book. His style reflects an enthusiasm to relate a scientific subject to the nonexpert reader. The naturalist in the author is expressed throughout the book and particularly when he is writing about the few remaining areas of natural beauty such as Point Pelee, Point aux Pins, and Long Point Bay. I have not experienced such infectious enthusiasm for a subject since I read Sir Alister Hardy's "Great Waters."

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(R693)

ANNOUNCEMENTS/AVIS

International Conference on Biomanipulation of Natural and Artificial Freshwater Ecosystems

This conference will be held at Lake Kinneret, Tiberias, Israel, August 2–7, 1987, to bring together researchers in different areas of limnology, fisheries, and aquatic resource management for an exchange of information and ideas and to stimulate interest in biomanipulation of lakes, rivers, and reservoirs. The scope of the conference will include the control and management of water quality, plankton, benthos, macrophyte, and fish populations. The language for the conference is English.

For further information please contact Dr. Moshe Gophe, Chairman, Local Organizing Committee, The Yigal Allon Kinneret Limnological Laboratory, P.O. Box 345, Tiberias, Israel 14102.

VIth World Water Congress on Water Resources

Announcement and Call for Papers for the VIth International Water Resources Association World Congress on Water Resources to be held at the Ottawa Congress Centre, Ottawa, Ont., Canada, from May 29 to June 3, 1988. The overall theme for the Congress is "Water for World Development."

For information on abstracts, submissions, and attendance, please write to The Secretariat, VIth IWRA World Water Congress on Water Resources, University of Ottawa, 631 King Edward Avenue, Ottawa, Ont. K1N 6N5. Telephone: (613) 564-3902; telex: 053 3338.

1986 W. F. Thompson Award American Institute of Fishery Research Biologists

In recent years the American Institute of Fishery Research Biologists (AIFRB) has presented an award to the student writing the best paper *published* during the past three-year period. The award is the W. F. Thompson Award and the person receiving it would receive a certificate from AIFRB, as well as a cheque for U.S.$750.

The award committee of AIFRB is now receiving nominations for the 1986 W. F. Thompson Award which will cover *the period 1983–1985*. Papers can have multiple authors, but the student nominated for the award must be the principal author. Peers and professors can be listed as junior authors. Where a student receives the award and certificate for a multiple authored paper, the professor will receive a certificate acknowledging his role in the outstanding paper.

Papers should be concerned with freshwater or marine fisheries topics and problems, and may deal with biology or management problems. The papers are evaluated by the AIFRB award committee on the basis of originality, organization and development, and pertinence to current problems in fisheries. Nominations for papers should be received no later than 31 July 1986. A final decision will be announced by 29 August 1986. Five copies of each paper nominated should be sent to

Conférence internationale sur la biomanipulation des écosystèmes d'eau douce naturels et artificiels

Cette conférence, qui aura lieu au lac Kinneret (Tiberias, Israel) du 2 au 7 août 1987, vise à réunir des chercheurs de différents domaines de la limnologie, des pêches et de la gestion des ressources aquatiques pour leur permettre d'échanger de l'information et des idées et pour stimuler leur intérêt à l'endroit de la biomanipulation des lacs, des cours d'eau et des réservoirs. La conférence portera notamment sur le contrôle et la gestion de la qualité de l'eau, du plancton, du benthos, des macrophytes et des populations de poissons. La conférence se tiendra en anglais.

Pour de plus amples renseignements, prière de communiquer avec D[r] Moshe Gophe, président, Comité local d'organisation, Laboratoire limnologique Yigal Allon Kinneret, B.P. 345, Tiberias (Israel) 14102.

Dépôt des communications

Le VI[e] Congrès mondial sur les ressources en eaux se tiendra du 29 mai au 3 juin 1988 au Centre des congrès d'Ottawa, Ont., Canada. Il aura pour thème «L'eau au service du développement mondial».

Pour obtenir des renseignements sur les résumés, les présentations et la participation, écrire au Secrétariat, VI[e] Congrès mondial sur les ressources en eaux, Université d'Ottawa, 631, avenue King Edward, Ottawa (Ont.) K1N 6N5. Téléphone : (613) 564-3902; telex : 053 3338.

Prix W. F. Thompson de 1986 American Institute of Fishery Research Biologists

L'American Institute of Fishery Research Biologists (AIFRB) accorde depuis quelques années un prix à l'étudiant qui est l'auteur de la meilleure communication *publiée* au cours des trois dernières années. Ce prix, le W. F. Thompson Award, est accompagné d'un certificat de l'Institut et d'une bourse de 750 $ U.S.

Le comité chargé d'attribuer le prix accepte actuellement les mises en nomination pour le prix de 1986 qui porte sur *la période de 1983 à 1985*. Une communication peut avoir plusieurs auteurs, mais l'étudiant mis en nomination doit en être le principal auteur. Des collègues et des professeurs peuvent cependant en être les auteurs secondaires. Quand un étudiant reçoit le prix pour une communication à plusieurs auteurs, un certificat est alors décerné au professeur afin de souligner son rôle dans la publication d'une communication de qualité supérieure.

Les communications doivent porter sur des questions ou des problèmes touchant les pêches en eau douce ou en mer et peuvent traiter de questions ayant trait à la biologie ou à la gestion. Les articles sont évalués par le comité de l'Institut chargé de l'attribution du prix qui se base sur leur originalité, leur présentation et développement et leur pertinence dans le

Dr. W. (Tony) Kwain, Chairman, AIFRB W. F. Thompson Award Committee, Ontario Ministry of Natural Resources, Lake Superior Fisheries Research Unit, P.O. Box 130, Sault Ste. Marie, Ont. P6A 5L5.

If there are questions in regard to the award, the Chairman can be reached at (705) 949-1231, extension 274.

contexte des problèmes affectant les pêches. Les nominations doivent être parvenues au comité au plus tard le 31 juillet 1986. La décision finale sera rendue publique le 29 août 1986. Les communications doivent être présentées en cinq exemplaires et expédiées à l'adresse suivante : Dr. W. (Tony) Kwain, Chairman, AIFRB W. F. Thompson Award Committee, Ministère des Ressources naturelles de l'Ontario, Lake Superior Fisheries Research Unit, C.P. 130, Sault Ste. Marie (Ont.) P6A 5L5.

Il est possible d'obtenir des renseignements supplémentaires en communiquant avec le président du comité au (705) 949-1231, poste 274.

COMING NEXT MONTH/À PARAÎTRE LE MOIS PROCHAIN

Articles

Algal competition for phosphorus: the influence of zooplankton and fish
H. Reinertsen, A. Jensen, A. Langeland, and Y. Olsen

Influence of starvation and subsequent feeding on body composition and energy reserves in the prawn *Pandalus platyceros* **J. N. C. Whyte, J. R. Englar, B. L. Carswell, and K. E. Medic**

Digestibility and utilization of canda meal in practical-type diets for rainbow trout (*Salmo gairdneri*)
J. W. Hilton and S. J. Slinger

Current velocity in streams and the composition of benthic algal mats **M. A. Reiter and R. E. Carlson**

Effects of environmental variables on growth rates and physiological characteristics of Lake Superior phytoplankton **C. Nalewajko and D. Voltolina**

Experimental study of the impacts of bluegill (*Lepomis macrochirus*) and largemouth bass (*Micropterus salmoides*) on pond community structure
K. D. Hambright, R. J. Trebatoski, R. W. Drenner, and D. Kettle

Seasonal inorganic carbon and nitrogen transport by phytoplankton in an arctic lake
S. C. Whalen and V. Alexander

Dynamics of discovery and exploitation: the case of the Scotian Shelf groundfish fisheries
P. M. Allen and J. M. McGlade

Phosphorus release processes in nearshore southern Lake Michigan **M. A. Quigley and J. A. Robbins**

Delayed metamorphosis with reduced larval growth in a coral reef fish (*Thalassoma bifasciatum*)
B. C. Victor

An experimental and statistical approach to the question: Is CPUE an index of abundance?
L. J. Richards and J. T. Schnute

Comparisons of herring otoliths using Fournier series shape analysis
J. L. Bird, D. T. Eppler, and D. M. Checkley, Jr.

Microhabitat–preference curves of blackfly larvae (Diptera: Simuliidae): a comparison of three estimation methods **A. Morin, P.-P. Harper, and R. H. Peters**

Variation in male parr maturation within and among populations of Atlantic salmon, *Salmo salar*
R. A. Myers, J. A. Hutchings, and R. J. Gibson

Precipitation chemistry and chemical limnology of fertilized and natural lakes at Saqvaqjuac, N.W.T.
H. E. Welch and J. A. Legault

Winter hydrography and plankton distribution on the southern British Columbia continental shelf
A. C. Thomas and W. J. Emery

Carbon flow in a tundra stream ecosystem **B. J. Peterson, J. E. Hobbie, and T. L. Corliss**

Benthic bacterial biomass and production in two blackwater rivers
S. Findlay, J. L. Meyer, and R. Risley

Reviews/Synthèses

Theory, practice, and effects of *Mysis relicta* introductions to North American and Scandinavian lakes
D. C. Lasenby, T. G. Northcote, and M. Fürst

Notes

Influence of pH, aluminum, and organic matter on stream invertebrates
T. M. Burton and J. W. Allan

Estimating fork lengths of fathead minnows, *Pimephales promelas*, from measurement of pharyngeal arches
D. B. McIntyre and F. J. Ward

Patterns and rates of ammonia excretion by juvenile American lobsters, *Homarus americanus*, fed casein- and crab protein-based diets **C. M. Hawkins, J. D. Castell, and V. Leroyer**

Perspectives

Decline in natural fisheries — a genetic analysis and suggestion for recovery **G. W. Wohlfarth**

Letters and comments/Lettres et commentaires

Comment — **G. H. Winters**
Reply — **S. E. McGladdery**

Book reviews/Comptes rendus

Seafood toxins (edited by Edward P. Ragelis) — **J. L. C. Wright**
Concepts in marine pollution measurements (edited by H. Harris) — **J. Vandermeulen**

Obituary/Nécrologie

Paul Frederick Elson, 1910–1985 C. J. Kerswill **1082–1083**

Letters and comments/Lettres et commentaires

Some remarks on "Determinants of catching power in the British Columbia purse seine fleet" by Hilborn and Ledbetter D. A. Fournier **1084–1086**
Response to Fournier R. Hilborn **1086–1088**
Letter to the editor S. J. Smith and P. E. J. Green **1088–1092**
In defense of the Instream Flow Incremental Methodology D. J. Orth and O. E. Maughan **1092–1093**
Reply to "In defense of the Instream Flow Incremental Methodology" by D. J. Orth and O. E. Maughan D. Mathur, W. H. Bason, E. J. Purdy, Jr., and C. A. Silver **1093–1094**

Book reviews/Comptes rendus

FAO species catalogue. Vol. 4. Sharks of the world: an annotated and illustrated catalogue of shark species known to date (L. J. V. Compagno) G. Dingerkus **1095–1097**
Erie: the lake that survived (Noel M. Burns) J. H. Leach **1097**

Announcements/Avis **1098–1099**
Coming next month/À paraître le mois prochain **1100–1101**

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 43, No. 5, May 1986 **Volume 43, n° 5, mai 1986**

Articles

Laboratory feeding and growth of juvenile sablefish, *Anoplopoma fimbria* J. M. Shenker and B. L. Olla **930–937**
Dietary change during development in the freshwater calanoid copepod *Epischura lacustris* Forbes P. Chow-Fraser and C. K. Wong **938–944**
Experimental infection and pathogenesis of viral erythrocytic necrosis (VEN) in Atlantic cod, *Gadus morhua* P. W. Reno, K. Kleftis, S. W. Sherburne, and B. L. Nicholson **945–951**
Diel vertical movements and feeding activity of zooplankton in St. Georges Bay, N.S., using net tows and a newly developed passive trap G. C. Harding, W. P. Vass, B. T. Hargrave, and S. Pearre, Jr. **952–967**
Régime alimentaire et principaux facteurs influençant l'alimentation des larves de capelan (*Mallotus villosus*), d'éperlan (*Osmerus mordax*) et de hareng (*Clupea harengus harengus*) dans un estuaire partiellement mélangé R. Courtois et J. J. Dodson **968–979**
Different adaptation strategies of Atlantic salmon (*Salmo salar*) populations to extreme climates with special reference to some cold Norwegian rivers A. J. Jansen and B. O. Johnsen **980–984**
Population structure, ecological segregation, and reproduction in a population of Arctic char (*Salvelinus alpinus*) from Lake Tasersuag, Greenland F. F. Riget, K. H. Nygaard, and B. Christensen **985–992**
Long-term dynamics of three crayfish species in Trout Lake, Wisconsin D. M. Lodge, T. K. Kratz, and G. M. Capelli **993–998**
Model of monthly marine growth and natural mortality for Babine Lake sockeye salmon (*Oncorhynchus nerka*) D. J. Furnell and J. R. Brett **999–1004**
Factors influencing the microdistribution of two sympatric species of Plecoptera: an experimental study D. D. Williams **1005–1009**
Cortisol, aldosterone, and leucocytes in the stress response of bottlenose dolphins, *Tursiops truncatus* C. A. Thomson and J. R. Geraci **1010–1016**
Physiological responses to acid stress in crayfish (*Orconectes*): haemolymph ions, acid–base status, and exchanges with the environment C. M. Wood and M. S. Rogano **1017–1026**
Selected articles on fish genetics: introduction J. M. McGlade **1027**
Fish antifreeze proteins: recent gene evolution G. K. Scott, G. L. Fletcher, and P. L. Davies **1028–1034**
A distance constraint model for the prediction of protein tertiary structure E. A. D. Foster **1035–1044**
Evolutionary relationship of a fish C type lactate dehydrogenase to other vertebrate lactate dehydrogenase isozymes R. H. Rehse and W. S. Davidson **1045–1051**
Evolution of multifunctionalism in enzymes: specialist versus generalist strategies J. McGlade and P. Allen **1052–1058**
Artificial selection on growth and correlated selection on competitive behaviour in fish R. W. Doyle and A. J. Talbot **1059–1064**

Notes

An empirical model to predict in situ grazing rates of *Diaptomus minutus* Lilljeborg on small algal particles P. Chow-Fraser **1065–1070**
Spatial distribution of entrained fish larvae in a power plant discharge canal D. J. Jude, P. J. Mansfield, S. F. DeBoe, and F. J. Tesar **1070–1074**
Spatial correlations of transferrin allele frequencies in Atlantic salmon (*Salmo salar*) populations from North America E. Verspoor **1074–1078**
Changes in lengths of fixed and preserved young freshwater fish J. K. Leslie and J. E. Moore **1079–1081**

(continued inside back)

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